FORMULATION AND *IN VITRO* CHARACTERISATION OF GLIPIZIDE LOADED MUCOADHESIVE GELATIN MICROSPHERES

A Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY IN PHARMACEUTICS

Submitted by

Reg No.261211259

Under the guidance of

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April - 2014

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CERTIFICATE

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Place: Chennai-03.

Date: (Dr.A.Jerad Suresh)

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Place: Chennai-03.

Date: (Dr.K.Elango)

DEDICATED TO MY BELOVED PARENTS AND VINNU

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LIST OF ABBREVATIONS

GI	Gastrointestinal
PMMA	Poly methyl acrylate
°C	Degree centigrade
h	Hours
min	Minutes
rpm	Rotations per minute
µm	Micrometer
o/w	Oil in water
SLS	Sodium lauryl sulphate
w/v	Weight by volume
HAP	Hydroxy appatite
w/w	Weight by weight
EVA	Ethyl vinyl acetate
DCM	Dichloromethane
DM	Diabetes mellitus
IDDM	Insulin dependent diabetes mellitus
NIDDM	Non insulin dependent diabetes mellitus
mg	Milligram
ADA	American diabetes association
dL	Deciliter
HDL	High density lipoprotein
ATP	Adenosine triphosphate
SMBG	Self monitoring blood glucose
ml	Milliliter
g/mol	Grams by mole
L	Litre
DDS	Drug delivery system
FTIR	Fourier transform infra red
UV	Ultraviolet

g	Gram
nm	Nanometer
cm	Centimeter
EE	Entrapment efficiency
SEM	Scanning electron microscope
RH	Relative humidity
ICH	International council on Harmonization
SR	Sustained release
SD	Standard deviation

CONTENTS

1. INTRODUCTION

1.1 ORAL CONTROLLED DRUG DELIVERY SYSTEMS[1,2]

Oral drug delivery is the most widely utilized route of administration among all the routes that have been explored for systemic delivery of drugs. Oral route is considered most natural, uncomplicated, convenient and safe due to its ease of administration, patient acceptance and cost effective manufacturing process.

Pharmaceutical products designed for oral delivery are mainly conventional drug delivery systems, which are designed for immediate release of drug for rapid absorption. These immediate release dosage forms have some limitations such as:

1. Drugs with short half-life require frequent administration leading to poor patient compliance.
2. A typical peak-valley plasma concentration-time profile is obtained, which makes attainment of steady state condition difficult.
3. The unavoidable fluctuations in the drug concentration.

In order to overcome these drawbacks of conventional drug delivery systems, several technical advancements have led to the development of controlled drug delivery system that could revolutionize method of medication and provide a number of therapeutic benefits.[1, 2]

Controlled release drug delivery systems are more preferred over conventional drug delivery systems, since they are associated with several advantages like:

1. Reduced dosing frequency.
2. Reduced fluctuation in plasma drug levels.
3. Increased patient compliance.
4. Maximum utilization of drug.
5. Minimal local and systemic side effects.
6. Reduced health care costs.

However these systems are marked by several disadvantages like:

1. Dose dumping.
2. Reduced potential for accurate dose adjustment.
3. Need for additional patient education.
4. Possible reduction in systemic availability.
5. Does not permit to termination of therapy in emergency.
6. Stability problems.

Oral controlled release drug delivery is a drug delivery system that provides continuous oral delivery of drugs at predictable and reproducible kinetics for a predetermined period throughout the course of GI transit. All the pharmaceutical products formulated for systemic delivery via the oral route of administration, irrespective of the mode of delivery (immediate, sustained or controlled release) and the design of dosage form (solid, dispersion or liquid), must be developed within the intrinsic characteristics of GI physiology. Therefore the scientific framework required for the successful development of oral drug delivery systems consists of basic understanding of (i) physicochemical, pharmacokinetic and pharmacodynamic characteristics of the drug (ii) the anatomic and physiologic characteristics of the GI tract and (iii) physicochemical characteristics and the drug delivery mode of the dosage form to be designed.[1]

Amongst the various oral controlled drug delivery systems mucoadhesive microspheres have generated much interest among researchers around the world. The microspheres offer number of benefits including reducing stress resulting from restraint, stability, handling and dosing, avoiding expensive and difficult drug administration procedures.

1.2 MUCOADHESIVE DRUG DELIVERY SYSTEMS [3]

1.2.1 Definition

The term “mucoadhesion” was coined for the adhesion of the polymers with the surface of the mucosal layer. Bioadhesion is a phenomenon in which two materials at least one of which is biological and are held together by means of interfacial forces.

Mucoadhesion is defined as the attachment of synthetic or biological macromolecules to the biological surface, which can be epithelial tissue or the mucus coat on the surface of tissue.[3]

1.2.2 Advantages

Mucoadhesive drug delivery systems have three distinct advantages when compared to conventional dosage forms.

1. The mucoadhesive systems, which are readily localized in the applied region can enhance or improve the bioavailability of drugs.
2. These dosage forms can facilitate the intimate contact with underlying absorption surface resulting in a better absorption.
3. They can prolong residence time at the site of application to permit once or twice a day dosing.

1.3 MECHANISM OF MUCOADHESION [4]

A General Mechanism of Mucoadhesive Drug Delivery system is show in Figure 1

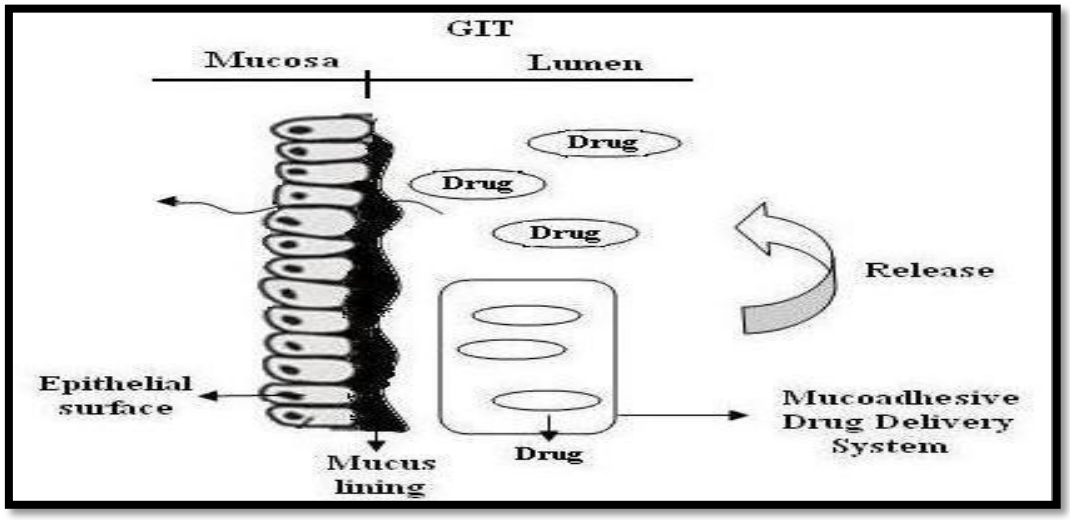


Figure 1- Mechanism of Mucoadhesion

Certain theories have been proposed to explain the fundamentals of adhesion,

1.3.1 Electronic theory[5]

According to this theory, electron transfers occur upon contact of adhesive polymer with a mucus glycoprotein network because of difference in their electronic structures. This results in the formation of electrical double layer at the interface e.g. Interaction between positively charged polymers chitosan and negatively charged mucosal surface which

becomes adhesive on hydration and provides an intimate contact between a dosage form and absorbing tissue.

1.3.2 Absorption theory

According to this theory, after an initial contact between two surfaces, the material adheres because of surface force acting between the atoms in two surfaces. Two types of chemical bonds resulting from these forces can be distinguished as primary chemical bonds of covalent nature and secondary chemical bonds having many different forces of attraction, including electrostatic forces, Vanderwals forces, hydrogen and hydrophobic bonds.

1.3.3 Diffusion theory

According to this theory, the polymer chains and the mucus mix to a sufficient depth to create a semi permanent adhesive bond. The exact depth to which the polymer chain penetrates the mucus depends on the diffusion coefficient and the time of contact. The diffusion coefficient in terms depends on the value of molecular weight between crosslinking and decreases significantly as the cross linking density increases.

1.3.4 Wetting theory

The wetting theory postulates that if the contact angle of liquids on the substrate surface is lower, then there is a greater affinity for the liquid to the substrate surface. If two substrate surfaces are brought in contact with each other in the presence of the liquid, the liquid may act as an adhesive among the substrate surface.

1.3.5 Cohesive theory

The cohesive theory proposes that the phenomena of bioadhesion are mainly due to intermolecular interaction amongst like molecule. Based upon the above theories, the process of bioadhesion can broadly be classified into two categories namely chemical (electron and absorption theory) and physical (wetting, diffusion and cohesive theory).

1.4 POLYMERS USED IN MUCOADHESIVE DRUG DELIVERY SYSTEMS[5]

Mucoadhesive polymers are water soluble as well as water insoluble polymers, which are swellable networks, joined by cross-linking agents. These polymers possess optimal polarity to make sure that they permit sufficient wetting by the mucus and optimal

fluidity that permits the mutual adsorption and interpenetration of polymer and mucus to take place. Mucoadhesive polymers that adhere to the mucin-epithelial surface can be conveniently divided into three broad classes.

1. Polymers that become sticky when placed in water and owe their mucoadhesion to stickiness.
2. Polymers that adhere through hydrogen and hydrophobic bonding interactions.
3. Polymers that bind to specific receptor site.

1.4.1Characteristics of Mucoadhesive Polymer

The following characteristics are believed to be essential for exhibiting the good Mucoadhesive properties:

(1) Strong hydrogen bonding groups,
(2) Strong anionic charges,
(3) High molecular weight,
(4) Sufficient chain flexibility and
(5) Surface energy properties favoring spreading onto mucus.

1.4.2 Hydrophilic polymers

The polymers within this category are soluble in water. Matrices developed with these polymers swell when put into an aqueous media with subsequent dissolution of the matrix. The polyelectrolytes extend greater mucoadhesive property when compared with neutral polymers. Anionic polyelectrolytes, e.g. poly (acrylic acid) and carboxymethyl cellulose have been extensively used for designing mucoadhesive delivery systems due to their ability to exhibit strong hydrogen bonding with the mucin present in the mucosal layer. Gelatin, chitosan provides an excellent example of cationic polyelectrolyte, which has been extensively used for developing mucoadhesive polymer due to its good biocompatibility and biodegradable properties.

1.4.3 Hydrogels

Hydrogels can be defined as three-dimensionally cross linked polymer chains which have the ability to hold water within its porous structure. The water holding capacity of the

hydrogels is mainly due to the presence of hydrophilic functional groups like hydroxyl, amino and carboxyl groups. Hydrogels prepared by the condensation reaction of poly (acrylic acid) and sucrose indicated an increase in the mucoadhesive property with the increase in the cross linking density and was attributed to increase in the poly (acrylic acid) chain density per unit area.

Acrylates have been used to develop mucoadhesive delivery systems which have the ability to deliver peptide bioactive agents to the upper small intestine region without any change in the bioactivity of the peptides. Wheat germ agglutinin helped in improving the intestinal residence time of the delivery system by binding with the specific carbohydrate moieties present in the intestinal mucosa.

1.4.4 Thiolated polymers

The presence of free thiol groups in the polymeric skeleton helps in the formation of disulphide bonds with that of the cysteine-rich sub-domains present in mucin which can substantially improve the mucoadhesive properties of the polymers e.g. poly (acrylic acid) and chitosan) in addition to the paracellular uptake of the bioactive agents. Various thiolated polymers include chitosan–iminothiolane, poly (acrylic acid)–cysteine, poly (acrylic acid)–homocysteine, chitosan–thioglycolic acid, Chitosan–thioethylamidine, alginate–cysteine, poly (methacrylic acid)–cysteine and sodium carboxymethylcellulose–cysteine

1.4.5 Lectin-based polymers

Lectins are proteins which have ability to reversibly bind with specific sugar carbohydrate residues and are found in both animal and plant kingdom. The specific affinity of lectins towards sugar or carbohydrate residues provides them with specific cyto-adhesive property and is being explored to develop targeted delivery systems. Lectins extracted from legumes have been widely explored for targeted delivery systems.

1.5 MICROSPHERES [6,7]

Microspheres are spherical solid particles ranging in size from 1-1000 µm. They are spherical free flowing particles consisting of proteins or synthetic polymers which are biodegradable in nature. There are two types of microspheres;

- Microcapsules
- Micromatrices

Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micromatrices in which entrapped substance is dispersing throughout the microspheres matrix. Solid biodegradable microspheres incorporating a drug dispersed or dissolved through particle matrix have the potential for the controlled release of drug. They are made up of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products.

1.6 TYPES OF MICROSPHERES

1.6.1 Bioadhesive microspheres

Adhesion can be defined as sticking of drug to the membrane by using the sticking property of the water soluble polymers. Adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal, etc can be termed as bio adhesion. This kind of microspheres exhibit prolonged residence time at the site of application and produces better therapeutic effect.

1.6.2 Magnetic microspheres

This kind of delivery system is very much important which localises the drug to the disease site. In this, larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres like chitosan, dextran etc.

- Therapeutic magnetic microspheres: Are used to deliver chemotherapeutic agent to liver tumour. Drugs like proteins and peptides can also be targeted through this system.
- Diagnostic microspheres: Can be used for imaging liver metastasis and also can be used for distinguish bowel loops from other abdominal structures by forming nano size particles supramagnetic iron oxides.

1.6.3 Floating microspheres.

In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, if the system is floating on gastric content it increases gastric residence time and increases fluctuation in plasma concentration. Moreover it also reduces chances of

dose dumping and striking and produces prolonged therapeutic effect therefore reduces dosing frequencies. Drug (ketoprofen) given in this form.

1.6.4 Radioactive microspheres

Radio emobilisation therapy microspheres sized 0- 30 nm are of larger than capillaries and gets tapped in first capillary bed when they come across. They are injected to the arteries that lead to tumour of interest. Radioactive microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues. It differs from drug delivery system, as radio activity is not released from microspheres within a radioisotope typical distance and the different kinds of radioactive microspheres are α emitters, β emitters, γ emitters.

1.6.5 Polymeric microspheres

The different types of polymeric microspheres can be classified as followed they are biodegradable polymeric microspheres and synthetic polymeric microspheres.

1.6.5 (a) Biodegradable polymeric microspheres

Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible and also bioadhesive in nature. Biodegradable polymers prolongs the residence time when contact mucous membrane due to its high degree of swelling property with aqueous medium, results gel formation.

The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. The main drawback is in clinical use drug loading efficiency of biodegradable microspheres is complex and is difficult to control the drug release.

1.6.5 (b) Synthetic polymeric microspheres

The interest of synthetic polymeric microspheres are widely used in clinical application as bulking agent, fillers, embolic particles, drug delivery vehicles etc. and proved to be safe and biocompatible. But the main disadvantage of these kinds of microspheres, are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

Microspheres are usually are polymers. They are classified into two types:

- Synthetic Polymers
- Natural Polymers

Synthetic polymers are divided into two types.

a) Non- biodegradable polymers
   Poly methyl acrylate (PMMA), Acrolein, Glycidyl methacrylate, Epoxy polymers.

b) Biodegradable polymers
   Lactides, Glycolides& their copolymers, Poly alkyl cyanocrylates, Poly anhydrides

Natural polymers obtained from different sources like proteins, carbohydrates and chemically modified carbohydrates.

Proteins : Albumins, Gelatin, Collagen

Carbohydrates : Agarose, Carrageenan, Chitosan, Starch

Chemically modified carbohydrates : Poly (acryl) dextran, Poly (acryl) starch

Preparation of microspheres should satisfy certain criteria:

1. The ability to incorporate reasonably high concentration of the drug.
2. Stability of the preparation after synthesis with a clinically acceptable shelf life.
3. Controlled particle size and dispersibility in aqueous vehicles for injection.
4. Release of active reagent with a good control over a wide time scale.
5. Biocompatibility with a controllable biodegradability
6. Susceptibility to chemical modification.

1.7 METHOD OF PREPARATION[7]

1.7.1 Emulsion Solvent evaporation technique

In this technique the processes are carried out in a liquid manufacturing vehicle. The microcapsule coating is dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size

microcapsule. The mixture is then heated if necessary to evaporate the solvent for the polymer of the core material is disperse in the polymer solution, polymer shrinks around the core. If the core material is dissolved in the coating polymer solution, matrix – type microcapsules are formed. The core materials may be either water soluble or water insoluble materials. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous.

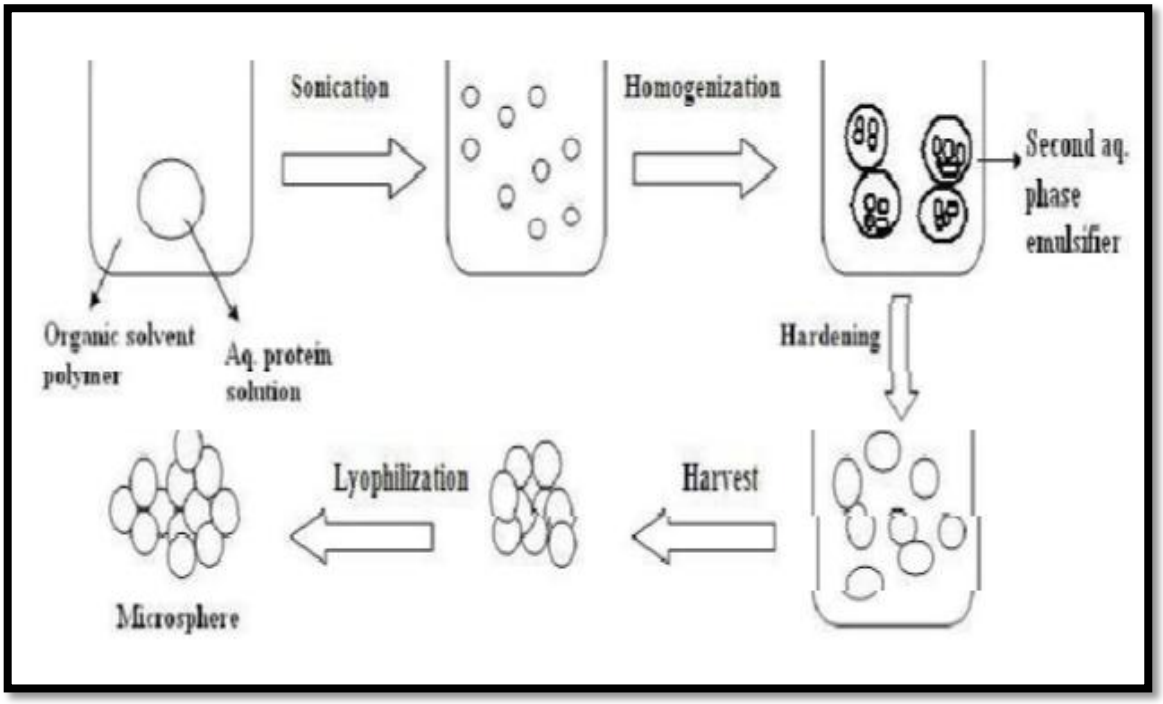


Figure 2- Solvent evaporation technique

1.7.2 Emulsion- Solvent Diffusion Technique

In order to improve the residence time in colon floating micro particles of ketoprofen were prepared using emulsion solvent diffusion technique. The drug polymer mixture is dissolved in a mixture of ethanol and dichloromethane (1:1) and then the mixture is added drop wise to sodium lauryl sulphate (SLS) solution. The solution is stirred with propeller type agitator at room temperature at 150 rpm for 1 h. Thus the formed floating microspheres were washed and dried in a dessicator at room temperature. The following micro particles were sieved and collected.

1.7.3 Emulsion Cross linking Method

In this method drug is dissolved in aqueous gelatine solution which is previously heated for 1 h at 40°C. The solution is added drop wise to liquid paraffin while stirring the mixture at 1500 rpm for 10 min at 35°C, results in w/o emulsion then further stirring is done for 10 min at 15°C.

The produced microspheres are washed respectively three times with acetone and isopropyl alcohol which then air dried, dispersed in 5mL of aqueous glutaraldehyde saturated toluene solution at room temperature for 3 h cross linking and then treated with 100ml of 10 mm glycerine solution containing 0.1% w/v tween 80 at 37°C for 10 min to block unreacted glutaraldehyde.

1.7.4 Multiple emulsion method

Multiple emulsion method involves formation of (o/w) Primary emulsion (non aqueous drug solution in polymer solution) and then addition of primary emulsion to external oily phase to form o/w/o emulsion followed by either addition of cross linking agent (glutaraldehyde) and evaporation of organic solvent.

Multiple emulsion method of preparation is ideal for incorporating poorly aqueous soluble drug, thus enhancing its bioavailability.

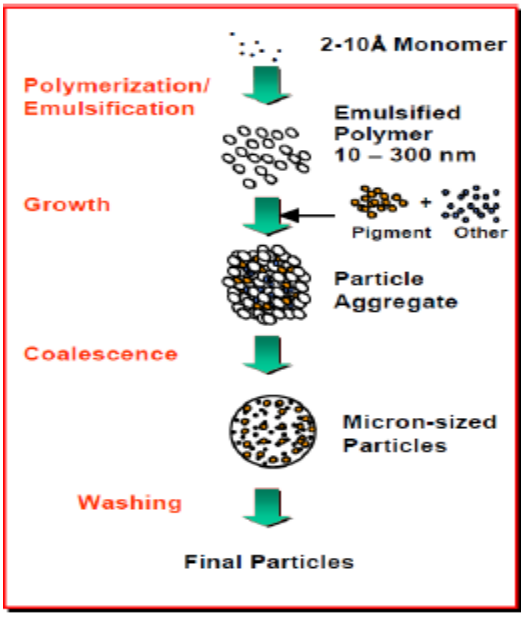


Figure 3- Multiple emulsion technique

1.7.5 Co-acervation method

This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Polylactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. The process variables are very important since the rate of achieving the coacervates determines the distribution of the polymer film, the particle size and agglomeration of the formed particles. The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer since as the process of microspheres formation begins the formed polymerize globules start to stick and form the agglomerates. Therefore the process variables are critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment.

1.7.6 Spray drying technique

In Spray drying, the polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporate instantaneously leading the formation of the microspheres in a size range 1-100µm. Microparticles are separated from the hot air by means of the cyclone separator while the trace of solvent is removed by vacuum drying. One of the major advantages of process is feasibility of operation under aseptic conditions. This process is rapid and this leads to the formation of porous microparticles.

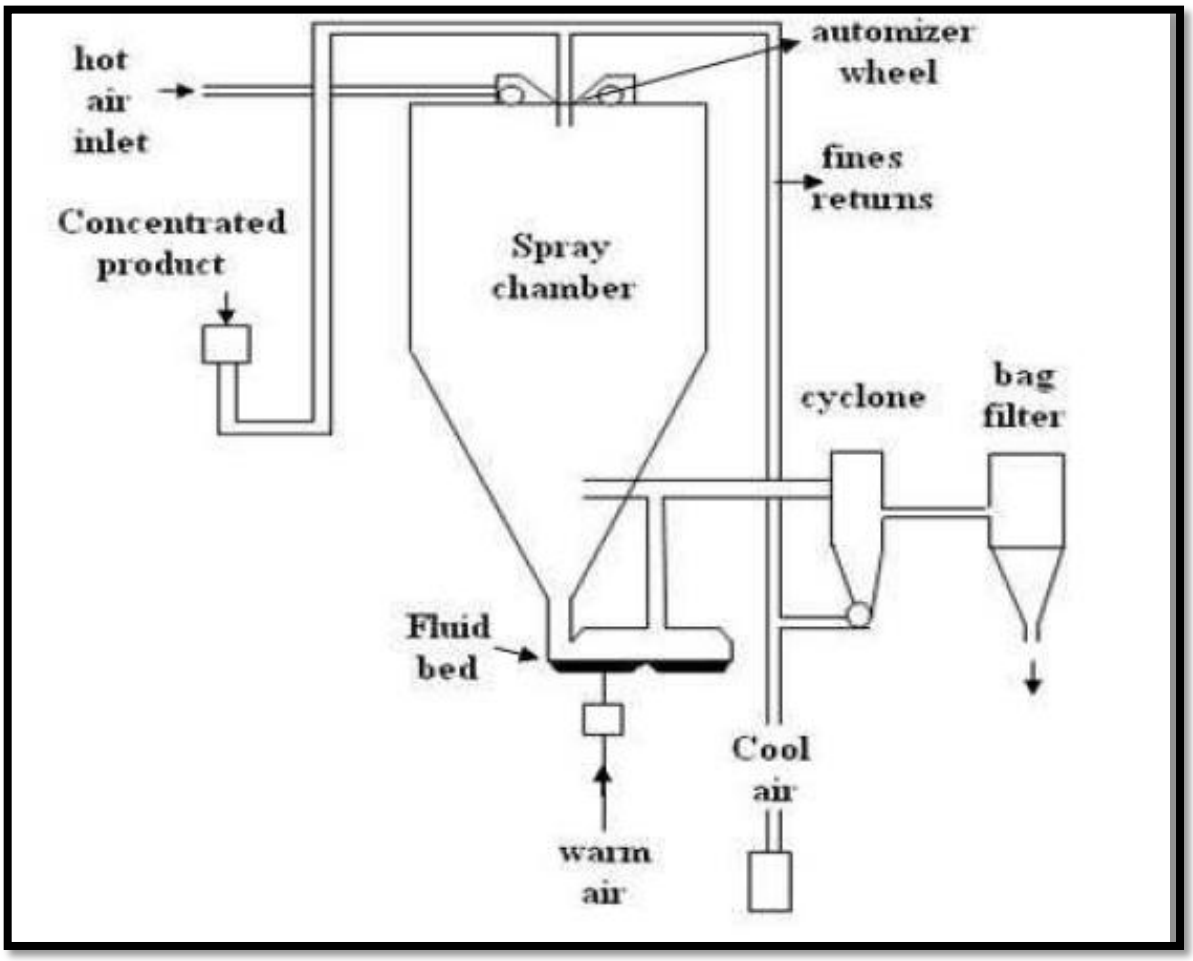


Figure 4- Spray drying technique

1.7.7 Ionic gelation

Alginate/ chitosan particulate system for diclofenac sodium release is prepared using this technique.25% (w/v) of diclofenac sodium is added to1.2 % (w/v) aqueous solution of sodium alginate. In order to get the complete solution stirring is continued and after that it is added drop wise to a solution containing Ca^{2+}/ Al^{3+} chitosan solution in acetic acid. Microspheres which were formed were kept in original solution for internal gelification followed by filtration for separation. The complete release is obtained at pH 6.4-7.4 but the drug did not release in acidic pH.

1.7.8 Hydroxy appatite (HAP) microspheres in sphere morphology

This method is used to prepare microspheres with peculiar spheres in sphere morphology microspheres were prepared by o/w emulsion followed by solvent evaporation.

At first o/w emulsion is prepared by dispersing the organic phase (Diclofenac sodium containing 5%w/w of EVA and appropriate amount of HAP) in aqueous phase of surfactant. The organic phase is dispersed in the form of tiny droplets which were surrounded by surfactant molecules; this prevented the droplets from co solvencing and helped them to as stay individual droplets. While stirring the DCM is slowly evaporated and the droplets solidify individually to become microspheres.

1.8 ENTRAPMENT OF DRUG IN MICROSPHERES[8]

The active component can be loaded by means of the physical entrapment, chemical linkage and surface absorption. The entrapment largely depends on the method of preparation and nature of the drug or polymer. The loading is carried out in pre-formed microspheres by incubating them with high concentration of the drug in a suitable solvent.

1.9 DRUG RELEASE KINETICS FROM MICROSPHERES[8]

Release of the active constituent is an important consideration in case of microspheres. Many theoretically possible mechanisms may be considered for the release of drug from the microparticulates.

1. Liberation due to polymer erosion or degradation,
2. Self diffusion through the pore,
3. Release from the surface of the polymer,
4. Pulsed delivery initiated by the application of an oscillating or sonic field.

The release profile from the microspheres depends on the nature of the polymer used in the preparation as well as on the nature of the active drug. The release of drug from both biodegradable as well as non biodegradable microsphere(s) is influenced by structure or micro-morphology of the carrier and the properties of the polymer itself. The drugs could be released through the microspheres by any of the three methods,

I. The osmotically driven burst mechanism,
II. Pore diffusion mechanism and
III. By erosion or the degradation of the polymer.

In osmotically driven burst mechanism, water diffuses into the core through biodegradable or non biodegradable coating, creating sufficient pressure that ruptures the membrane. The burst effect is mainly controlled by three factors the macromolecule/ polymer ratio, particle size of the dispersed macromolecule and the particle size of the microspheres.

The pore diffusion method is named so because as penetrating waterfront continue to diffuse towards the core. The dispersed protein/drug dissolves creating a water filled pore network through which the active principle diffuses out in a controlled manner. In case of the biodegradable polymers, the release is controlled by both the erosion as well as diffusion process.

The polymer erosion, i.e. loss of polymer is accompanied by accumulation of the monomer in the release medium. The erosion of the polymer begins with the changes in the microstructure of the carrier as water penetrates within it leading to the plasticization of the matrix. This plasticization of the matrix finally leads to the cleavage of the hydrolytic bonds. The cleavage of the bond is also facilitated by the presence of the enzyme (lysozymes) in the surroundings.

The erosion of the polymer may be either surfacial or it may be bulk leading to the rapid release of the drug active components. The rate and extent of water uptake therefore determines release profile of the system and depends on type of the polymer, porosity of the polymer matrix, protein drug loading etc.

1.10 FACTORS AFFECTING THE RELEASE [8]

Controlled release is an attainable and desirable characteristic for drug delivery systems. The factors affecting the drug release rate revolve around the structure of the matrix where the drug is contained and the chemical properties associated with both the polymer and the drug.

Conventional oral delivery is not rate controlled. A drug encapsulated in a slowly degrading matrix provides the opportunity for slower release effects, but polymer degradation is not the only mechanism for the release of a drug. The drug release is also

diffusion controlled as the drug can travel through the pores formed during sphere hardening. In some cases, drugs containing nucleophilic groups can cause increased chain scission of the polymer matrix, which also increases the rate of drug expulsion. Polymer molecular weight, drug distribution, polymer blending, crystallinity and other factors are important in manipulating release profiles.

1.11 FACTORS AFFECTING THE RELEASE FROM THE PARTICULATE SYSTEM [8]

DRUG

- Position in microspheres
- Molecular weight
- Physicochemical properties
- Concentration
- Interaction with matrix

MICROSPHERES

- Type of the matrix polymer
- Amount of the matrix polymer
- Size and density of the microspheres
- Extent of cross linking
- Denaturation or polymerization
- Adjuvant

ENVIRONMENT

- pH
- Polarity
- Presence of enzyme

1.12. SUITABLE DRUG CANDIDATES FORMICROSPHERES DRUG DELIVERY[8]

Various drugs have their greatest therapeutic effect when released at the targeted area of body, particularly when the release is prolonged in a continuous, controlled manner. Drugs delivered in this manner have a lower level of side effects and provide their

therapeutic effects without the need for repeated dosages or with a low dosage frequency. Sustained release in the stomach is also useful for therapeutic agents that the stomach does not readily absorb, since sustained release prolongs the contact time of the agent in the stomach or in the other part of the body, which is where absorption occurs and contact time is limited. Appropriate candidate for microsphere drug delivery system is that

a) Drugs that possess narrow absorption window in GI tract. e.g., riboflavin, levodopa.
b) Drugs that act locally in the stomach. e.g., antacids and misoprostol.
c) Drugs having poor bioavailability.

1.13 RELEASE TYPE OF MICROSPHERES [8]

A] Reservoir type system

Release from the reservoir type system with rate controlling membrane proceeds by first penetration of the water through the membrane followed by dissolution of the drug in the penetrating dissolution fluid. The dissolved drug after partitioning through the membrane diffuses across the stagnant diffusion layer. The release is essentially governed by the Fick's first law of diffusion.

B] Matrix system:

Release profile of the drug from the matrix type of the device critically depends on the state of drug whether it is dissolved or dispersed in the polymer matrix. In the case of the drug dissolved in the polymeric matrix, amount of drug, and the nature of the polymer (whether hydrophobic or hydrophilic) affect the release profile.

1.14 ROUTE TARGETING [8]

I. Oral route

The controlled release systems have been developed for oral administration. Oral route is also suggested for the delivery of the soluble antigens. The risk of dose dumping is minimized with this formulation, the smaller sized and high drug loaded particles show faster release.

II. Intranasal route

In this type of targeting, the microspheres are given at the surface of nasal mucosa by considering the mucocilliary clearance. The particle size range of microspheres for targeting the respiratory tract is given table no. 1.

Table 1- Particle size of the microspheres for targeting of specific area

Respiratory	**Required Particle size (µm)**
Nose	25-30
Throat	20-28
Pharynx	20-24
Larynx	15-20
Trachea	10-15
Bronchi	8-12
Bronchioles	8-10
Alveolar duct	5-8
Alveoli	4-8

III. Ocular route

The eye and the cornea are easily accessible targets. The washout effect, however, presents difficulties in retention of micro particulate drug carrier in the corneal sac. The rapid conversion of the particulate suspension to gel form reportedly leads to their longer retention in the eye.

1.15 CHARACTERIZATION PARAMETERS & METHODS [8]

Microspheres are characterized in the following given parameters:

Table 2-Parameters & method for microspheres characterization

S.No	Characterizations Parameters	Method
1	Particle size and shape	Light microscopy& SEM
2	Chemical analysis	Electron Spectroscopy
3	Degradation of polymer	FTIR
4	Density	Pychnometer
5	Determination of isoelectric point	Micro electrophoresis
6	Surface Carboxylic Acid Residue	Radioactive glycine
7	Surface amino acid residue	Radioactive 14c acetic acid conjugate.
8	Capture efficiency	UV spectroscopy
9	Release study	USP basket apparatus
10	Flow property	Angle of contact

1.16 APPLICATIONS [7]

1) Medical Applications

- ✓ Release of proteins, hormones and peptides over extended period of time.
- ✓ Gene therapy with DNA plasmids and also delivery of insulin.
- ✓ Vaccine delivery for treatment of diseases like hepatitis, influenza, pertusis, ricin toxoid, diphtheria, birth control.

- ✓ Passive targeting of leaky tumour vessels, active targeting of tumour cells, antigens by intraarterial/ intravenous application.
- ✓ Tumour targeting with doxorubicin and also treatments of leishmaniasis.
- ✓ Magnetic microspheres can be used for stem cell extraction and bone marrow purging.
- ✓ Used in isolation of antibodies, cell separation and toxin extraction by affinity chromatography.
- ✓ Used for various diagnostic tests for infectious diseases like bacterial, viral, and fungal.

2) Radioactive Microsphere's Applications

- ✓ Can be used for radioembolisation of liver and spleen tumours.
- ✓ Used for radiosynvectomy of arthiritis joint, local radiotherapy, interactivity treatment.
- ✓ Imaging of liver, spleen, bone marrow, lung and thrombus in deep vein thrombosis.

3) Other Applications

- ✓ Fluorescent microspheres can be used for membrane based technologies for flow cytometry, cell biology, microbiology, Fluorescent Linked Immuno-Sorbent Assay.
- ✓ Yttrium 90 can be used for primary treatment of hepatocellular carcinoma.

2. REVIEW OF LITERATURE

- **Behera BC *et al*[16]** .prepared Glipizide loaded polymethacrylate microspheres by solvent evaporation method.The mean particle size ranged from 400-660µm and the encapsulation efficiencies ranged from 40.27-86.67%.

- **Mukul Sengupta *et al*[17]**.formulated and evaluated ethyl cellulose microspheres of Glipizide by solvent evaporation method.

- **Arora Neha *et al*[18]**. evaluated the entrapment efficiency of Glipizide microspheres. The percentage entrapment efficiency of eudragit coated Glipizide microspheres with and without plasticizers diisobutylpthalate and dioctylpthalate were observed. The increase in concentration of polymer entrapment efficiency was studied.

- **Shailesh Lokhande *et al*[19]**. developed Glipizide containing microballoons for floating controlled drug delivery system. The microballoons were prepared by emulsion solvent diffusion method using enteric acrylic polymer dissolved in a mixture of dichloromethane and ethanol.

- **Gaikwad Abhijit *et al*[20]**. designed the Glipizide pellets using fluid bed coating method; Drug loaded pellets were coated with dispersion of Eudragit NE 30 D : Eudragit 230 D 55(80:20) upto 30% weight gain. The shape and surface morphology by scanning electron microscopy and it shows uniformity in the coating process.The prepared pellets exhibited prolonged drug release (>12 h) by altering the theoretical weight gain of pellets.

- **Sarode SM *et al*[21]**. formulated floating microspheres of Glipizide by emulsion solvent diffusion technique.The percentage drug entrapment was found to be 91%. Buoyancy of microspheres was found to be more than 40% with 12 h floating capacity.

- **Cristina Pirvu[22]**. evaluated the process of Xantinol nicotinate release from unreticulated and for differently reticulated gelatin microspheres.An increase in the

microspheres size was correlated with a decrease of both the swelling and the drug release rates and proposed a diffusional model for the delivery of xantinol nicotinate.

- **Suja C Jayan *et al*[23]**.designed gelatin microspheres of Salbutamol sulphate by Co-acervation phase separation method and characterized by optical microscope and scanning electron microscopy. The percentage drug entrapment wasupto 80% and microspheres sustained the drug release over a period of 8.5 h.

- **Jeevana *et al*[24]**. prepared and evaluated gelatin microspheres of Tramadol hydrochloride by single emulsion technique with entrapment efficiency of 97.2%, drug release for 12 h and maximum drug release of 99.79%.

- **Ohta *et al*[25]**. Generated Cisplatin-conjugated gelatin microspheres (GMSs) and to confirm the subsequent release of cisplatin *invitro*. The GMSs (1mg) were immersed in 50µl of a cisplatin solution (0.06, 0.15, 0.27, 0.30 or 0.54 mg ml^{-1}) at 38˚C to allow conjugation. The platinum concentration in the GMSs samples was investigated as a function of the concentration of cisplatin solution used in their preparation, number of immersions in cisplatin (1, 2, 3, 4 or 5) and the period of immersion in proportion to the concentration of cisplatin solution and the length or number of immersions in cisplatin *invitro* release tests demonstrate that the release rate (%) from GMSs after 1, 3, 6, 12 or 24 h was 4.8, 5.5, 7.6, 10,0 and 12.4 respectively.

- **Wakode R *et al*[26]**. prepared gelatin mcrospheres for topical delivery of Vitamin A palmitate. Gelatin microspheres were prepared using Co-acervation method and process was optimized using 2^3 factorial designs. Drug loaded microspheres were incorporated in carbopol gel for controlled delivery for 24 h. The drug entrapment 67% was achieved with gelatin: drug (1:2). Drug release from microspheres followed Higuchi kinetics while formulation showed zero order release profile.

- **Chauhan MK *et al*[27]**. evaluated the effect of natural crosslinker on release of gelatin microspheres loaded with Flurbiprofen. Gelatin microspheres crosslinked with different percent of crosslinker(0.25, 0.5, 1%) were prepared.The results suggested that the degree of crosslinking increased with increased concentration of Genipin and consequently retard the release of drug from the gelatin microspheres.

- **Rahisuddin *et al*[28]**. studied the effect of stabilizing solvent on the preparation of Nimesulide loaded gelatin microspheres. The gelatin microspheres were prepared by emulsion cross linking technique with glutaraldehyde as cross linking agent using various stabilizing agents like sesame oil, liquid paraffin and soybean oil. The entrapment efficiency and drug release were maximum with nimesulide.

- **Leucuta SE *et al*[29]**. developed Nifedipine embedded in a gelatin matrix to develop a prolonged release dosage form. The effects of polymer/drug ratio, size of the beads, crosslinking with formaldehyde and ethylcellulose coating of the gelatin microspheres on the *invitro* release rate of the drug were investigated.The *invitro* release kinetics of nifedipine from gelatin microspheres were mainly first-order; from formaldehyde hardened gelatin microspheres, complied with the diffusion model for a spherical matrix, and from coated gelatin microspheres, obeyed zero-order kinetics. On administration of a single oral dose of nifedipine-loaded hardened gelatin microspheres to volunteers, suggest that the preparation can be considered as a sustained release delivery system for nifedipine.

- **Maria Angela Vandelli *et al*[30]**. hypothesis suggested gelatin microspheres treated by micro-waves 250˚C for 10 min can be easily loaded with drug by soaking process avoiding drug degradation.

- **Huang-Chien Liang *et al*[31]**. Evaluated Genipin-crosslinked gelatin microspheres as a drug carrier for intramuscular administration and performed *invitro* and *invivo* studies.

- **Sivakumar M *et al*[32]**. prepared composite microspheres from bioactive ceramics such as coralline hydroxyapatite [CHA, Ca_{10} $(Po_4)_6(OH)_2$] granules and gelatin by the dispersion polymerization and the Gentamicin was incorporated by the absorption method. The particle size found to be 16mm. The thermal behavior of composite microspheres was studied using thermogravimetric analysis and differential scanning calorimetric analysis. The cumulative *in vitro* release profile of gentamicin from composite microspheres showed near zero order patterns.

- **Chowdary KPR *et al*[33]**.studied on the *invitro* and *invivo* evaluation of ethyl cellulose microspheres of Glipizide prepared by an industrially feasible emulsion-solvent evaporation technique and microspheres were investigated. These microspheres were found suitable for parenteral controlled release.

- **Mustafa SK *et al*[34]**. studied on the formulation of controlled release Glipizide pellets using pan coating method. The surface morphologies and the cross sectional investigations were evaluated in the polarizing light microscope. As a result the coated pellet formulations were found to yield of glipizide between 8 to 13 h.

- **Chowdary KPR *et al*[35]**. studied on the drug and *invitro* and *invivo* evaluation of mucoadhesive microcapsules of Glipizide for oral controlled release. Microencapsulation has been accepted as a process to achieve controlled release and drug targeting. The study is to develop characterize and evaluate mucoadhesive microcapsules of glipizide employing various mucoadhesive polymers for prolonged gastrointestinal absorption.

- **Senthil A *et al*[36]**. developed chitosan loaded mucoadhesive microspheres of Glipizide for treatment of type 2 diabetes mellitus. Both *invitro* and *invivo* evaluations were carried out. Microspheres were prepared by simple emulsification technique.

- **Uma Mahesh *et al*[37]**.formulatedgelatin microspheres containing Diclofenac sodium by Co-acervation phase separation procedure using different drug, gelatin and HPMC ratio.

Microspheres with low amount of gelatin and high amount of HPMC showed prolonged action in *in vitro* dissolution studies.

- **Ofokonsi KC *et al*[38]**. formulated and evaluated microspheres based on Gelatin- Mucin admixtures for the rectal delivery of Cefuroxime Sodium by emulsification cross linking method. The inclusion of S-mucin in the composition of microspheres has an enhancer effect on the release rectal bioavailability of Cefuroxime sodium.

3. RATIONALE OF THE STUDY [14,15]

Drug delivery systems that can precisely control the release rates have an enormous impact on the healthcare system. The controlled release DDS are mainly aimed at controlling the rate of drug delivery and sustaining the duration of therapeutic activity. Drug release from these systems should be at a desired rate, predictable and reproducible. Amongst the various approaches for oral controlled DDS, mucoadhesive microspheres have generated much interest among researchers around the world, due to its increased GI transit time.

The drug of choice, Glipizide, is an effective anti -diabetic drug particularly in Type II Diabetes (Non-insulin dependent diabetes mellitus). It is a second generation sulfonylurea that actually lowers the blood glucose level in human by stimulating the pancreatic cell and thereby releasing the insulin. It has a short biological half-life of 2-5 hours, which necessitates its administration in 2 or 3 doses of 2.5 to 20 mg[14], to a maximum of 40 mg per day. Thus, the development of controlled release dosage form would be clearly advantageous.

Gelatin, a natural polymer can be used for the preparation of microspheres by emulsion cross linking technique, because of its ability to increase its biological half life by adhering to the mucous membrane.

The present work was designed with an aim of formulating mucoadhesive microspheres of Glipizide using Gelatin as a biocompatible polymer for per-oral administration and to further evaluate the formulation characteristics, mucoadhesion properties and the release of glipizide from the resulting microspheres.

4. PLAN OF THE WORK

4.1 Plan of Work:

The present work is carried out to prepare and evaluate the microspheres Glipizide using Gelatin as polymer in various proportions. The following experimental protocol was therefore designed to allow a systemic approach to the study.

- ✓ Preformulation studies
- ✓ FTIR studies
- ✓ Construction of standard calibration curve
- ✓ Formulation of Cross linked microspheres
- ✓ Particle size analysis by microscopy
- ✓ Shape, surface characterization
- ✓ Percentage yield
- ✓ Entrapment efficiency
- ✓ Drug loading Capacity
- ✓ Bulk density
- ✓ True density
- ✓ Hausner's ratio
- ✓ Compressibility index
- ✓ Angle of repose
- ✓ Swelling ratio
- ✓ *In vitro* mucoadhesion study
- ✓ *In-vitro* release study
- ✓ Mechanism of release kinetics
- ✓ Comparative study with marketed formulation
- ✓ Stability study

5. DISEASE PROFILE

5.1 DIABETES MELLITUS[9,10,11,12]

Diabetes mellitus is a group of metabolic diseases characterized by elevated blood glucose levels(hyperglycemia) resulting from defects in insulin secretion, insulin action or both. Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source. Chronic hyperglycemia is associated with microvascular and macro vascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke. In 1997 an estimated 4.5% of the US population had diabetes. Direct and indirect health care expenses were estimated at $98 billion.

Characteristics of the Common Types of Diabetes

	Type 1	Type 2
Age	Childhood	Pubertal
Onset	Acute; severe	Mild-severe; often insidious
Insulin secretion	Very low	Variable
Insulin sensitivity	Normal	Decreased
Insulin dependence	Permanent	Temporary; may occur later
Racial/ethnic groups at increased risk	All (low in Asians)	African Americans, Hispanics, Native Americans, Asian/Pacific Islanders
Genetics	Polygenic	Polygenic
Proportion of those with diabetes	80%	10%-20%
Association: obesity	No	Strong
Acanthosis nigricans	No	Yes
Autoimmune etiology	Yes	No

Figure 5- Characterestics of Diabetes mellitus

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipaemia, negative nitrogen balance and sometimes ketonaemia. A wide spread pathological changes is thickening of capillary basemen membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency.

Two major types of diabetes mellitus are:

5.1.1 TYPE I: Insulin Dependent Diabetes Mellitus (IDDM), juvenile onset of diabetes mellitus

There is β-Cell destruction in pancreatic islets; majority of cases are autoimmune (type 1A) antibodies that destroys β-Cells are detectable in blood, but some are idiopathic type (type 1B) no β-cell antibody is found. In all type 1 case circulating insulin levels are low or very low, and patients are more prone to ketosis. This type is less common and has low degree of genetic predisposition. In type 1 diabetes, the body does not produce insulin, and daily insulin injections are required. Over 700,000 people in the United States have type 1 diabetes; this is 5-10% of all cases of diabetes mellitus.

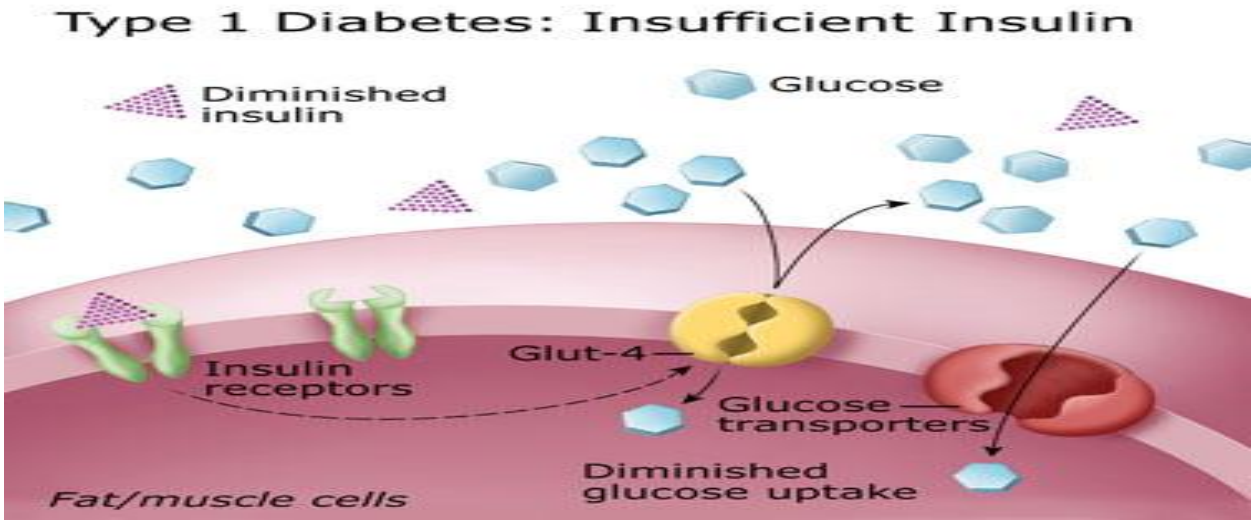


Figure 6 - Type 1 Diabetes mellitus

5.1.2 TYPE II: Non Insulin Dependent Diabetes Mellitus (NIDDM), maturity onset diabetes mellitus

There is no loss or moderate reduction in β-cell mass; insulin in circulation is low, normal or even high, no anti β-cell antibody is demonstrable; has a high degree of genetic predisposition; generally has late onset (past middle age). Over 90% cases are type 2 DM. Causes may be:

- Abnormality in gluco-receptor of β-cells so that they respond at higher glucose concentration or relative β-cell deficiency.
- Reduced sensitivity to peripheral tissues to insulin: reduction in number of insulin receptors, down regulation of insulin receptors. Many hypertensive's are hyperinsulinaemic, but normoglycaemic; exhibit insulin resistance associated with dyslipidaemia (metabolic syndrome). Hyperinsulinaemia per sec has been implicated in causing angiopathy
- Excess of hyperglycaemic hormones (glucagon, etc)/ Obesity cause relative insulin deficiency and the β-cells lag behind.

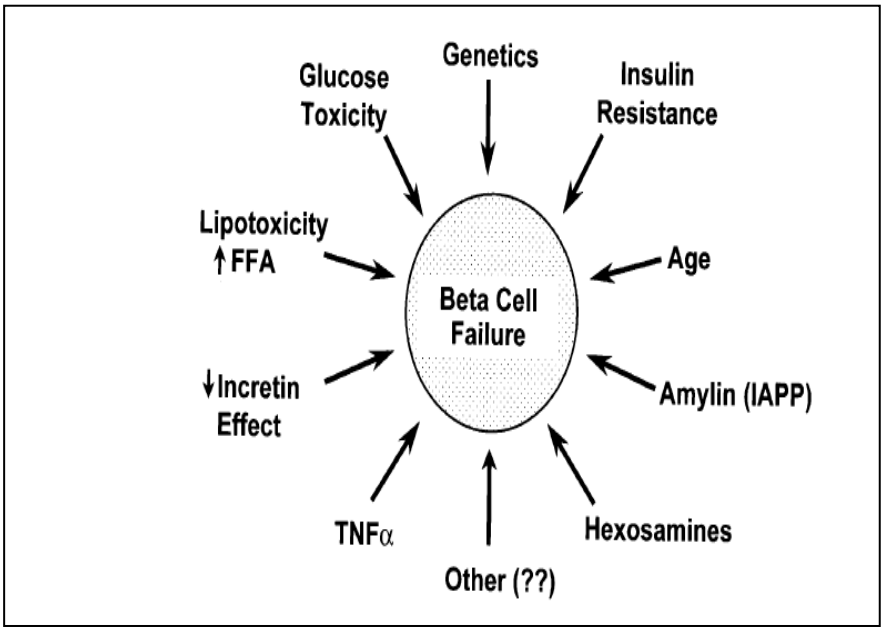


Figure 7 - Causes for beta cell failure

Type 2 diabetes is the result of failure to produce sufficient insulin and insulin resistance. Elevated blood glucose levels are managed with reduced food intake, increased physical activity, and eventually oral medications or insulin.

Type 2 diabetes is believed to affect more than 15 million adult Americans, 50% of whom are undiagnosed. It is typically diagnosed during adulthood. However with the increasing incidence of childhood obesity and concurrent insulin resistance, the number of children diagnosed with type 2 diabetes has also increased worldwide.

5.1.3 Contributing factors

– Obesity
– Age (onset of puberty is associated with increased insulin resistance)
– Lack of physical activity
– Genetic predisposition
– Racial/ethnic background (African American, Native American, Hispanic and Asian/ Pacific Islander)
– Conditions associated with insulin resistance (e.g., polycystic ovary syndrome)

5.1.4 Outcomes

Diabetes has significant associated morbidity and mortality. Patients with diabetes have a 2 to 4 fold increase in the risk of both cardiovascular and cerebrovascular disease, resulting in an increased mortality rate among patients with diabetes compared to the general population. Microvascular complications also occur including retinopathy, nephropathy and neuropathy and these can progress to the end-stage outcomes of blindness, renal failure and amputation.

Diabetes is the leading cause of new cases of blindness in adults ages 20-74 and the leading cause of end stage kidney disease in the U.S. seventy percent of non-traumatic lower extremity amputations occur in patients with diabetes. The morbidity and mortality of diabetes are higher for minorities than for Caucasians.

5.1.5 Screening

- The American Diabetes Association (ADA) recommends that screening be considered at least at 3-year intervals beginning at age 45. Screening individuals with risk factors for diabetes should be considered at earlier ages.

- Individuals with hypertension (>135/80) should be screened for diabetes (USPSTF level B recommendation). In adults who have hypertension and diabetes, lowering blood pressure below conventional target values reduces the incidence of cardiovascular events and cardiovascular mortality and justifies screening.

- Screening may be reasonable for other at-risk subjects (e.g., those with obesity, history of gestational diabetes mellitus, family history, and high-risk ethnic minorities). Based on expert opinion the ADA recommends considering earlier or more frequent screening for those with other risk factors including family history, physical inactivity, minority ethnicity, previously identified impaired fasting glucose or impaired glucose tolerance, a history of HDL cholesterol ≤ 35 mg/dL, and/or a triglyceride level of ≥ 250 mg/dL, polycystic ovarian disease, or a history of vascular disease.

- Women who have had gestational diabetes mellitus (GDM) should be screened for diabetes, as about 50% will have type 2 diabetes within 10 years.

5.1.6 Treatment

❖ **Glucose Lowering Therapy**

It is best to treat type 2 diabetes as vigorously as possible to avoid or delay the long term consequences of elevated blood glucose levels, high blood pressure, and dyslipidemia. Treatment focuses on discovering the most effective method to lower blood glucose levels, whether it is lifestyle modifications, insulin therapy, oral agents, or any combination of these factors. The diabetes team must work with the teen and the family to educate them about the importance of good control and to make the necessary adjustments in treatment every 4-6 weeks until acceptable control is achieved.

• At diagnosis, teens with type 2 diabetes who are acutely ill with significant hyperglycemia (>300mg/dl) and ketosis require insulin therapy. Insulin regimens are similar to those for teens with type1 diabetes. In the less ill teen, initial treatment with medical nutrition therapy and exercise or glucose lowering oral agent may be appropriate. In both circumstances, target blood glucose goals are similar to those with type 1 diabetes and treatment recommendations may change depending on blood glucose control.

• Glucose-lowering oral agents may be effective with type 2 diabetes.

Glucose-Lowering Oral Agents Commonly Used for Treatment of Type 2 Diabetes.

Type of Agent	Mechanism of Action	Generic Names
Biguanides	Decrease hepatic glucose production, increase muscle insulin sensitivity	Metformin
Sulfonylureas	Increase insulin secretion	Glyburide Glipizide Glimepiride
Meglitinide	Short-term promotion of glucose-stimulated insulin secretion	Repaglinide
Glucosidase inhibitors	Decrease digestion and absorption of carbohydrate	Acarbose Miglitol
Thiazolidenediones	Increase insulin action in muscle, adipose tissue and probably the liver	Rosiglitazone Pioglitazone

Figure 8 - Glucose lowering agents

❖ **Sulfonylureas**

Sulfonylureas lower serum glucose by increasing insulin secretion. Sulfonylureas are traditionally used as first line agents in type 2 diabetes.

Insulin secretagogues stimulate insulin secretion by interacting with the ATP-sensitive potassium channel on the beta cells. These drugs are most effective in individuals with type 2 DM of relatively recent onset (<5 years), who have residual endogenous insulin production.

First-generation sulfonylureas chlorpropamide, tolazamide, tolbutamide; have a longer half-life, a greater incidence of hypoglycemia, more frequent drug interactions, and are now rarely used.

Second-generation sulfonylureas have a more rapid onset of action and better coverage of the postprandial glucose rise, but the shorter half-life of some agents may require more than once-a-day dosing.

Sulfonylureas reduce both fasting and postprandial glucose and should be initiated at low doses and increased at 1 to 2 week intervals based on SMBG. In general, sulfonylureas increase insulin acutely and thus should be taken shortly before a meal; with chronic therapy, though, the insulin release is more sustained. Glimepiride and glipizide can be given in a single daily dose and are preferred over Glyburide.

Repaglinide and nateglinide are not sulfonylureas but also interact with the ATP-sensitive potassium channel. Because of their short half-life, these agents are given with each meal or immediately before to reduce meal-related glucose excursions.

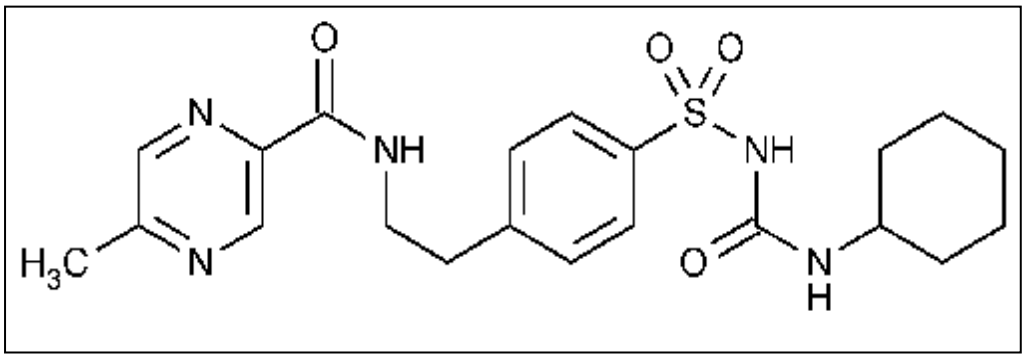
For patients with any renal impairment, glipizide is preferred. Severe hypoglycemia can occur in patients with significant renal impairment. Patients are typically treated with a second-generation sulfonylurea starting at a low dose. Dose increments may be made every two weeks.

6. DRUG PROFILE[13]

IUPAC Name

Glipizide is 1- cyclohexyl-3-[[4-2-[[5-methylpyrazine-2-yl) carbonyl]amino]ethyl]phenyl]sulphonyl]urea

Chemical structure



Molecular formula $C_{21}H_{27}N_5O_4S$

Molecular weight 445.5g/mol

BCS Classification Class-2 (High Permeability & low solubility)

Description A white or almost white, crystalline powder

Solubility Practically insoluble in water, sparingly soluble in acetone and soluble in methylene chloride (Dichloromethane), chloroform, dimethyl formamide.

Dose 5-20 mg twice or once daily

Category Oral hypoglycaemic agent

Mode of action Mechanism of action is produced by partially blocking potassium channels in the β cells of islets of langerhans.

Contra indications Glipizide is contraindicated in patients with

Known hypersensitivity to glipizide or any excipients in GIT tablets

Type 1 Diabetes, diabetic ketoacidosis with or without coma. This condition should be treated with insulin.

Pharmacokinetic Properties

Duration: 12-24 h.

Absorption: Rapid and complete; delayed with food.

Distribution: 10-11 L.

Protein binding: 98% to 99%; primarily to albumin.

Time to peak: 1-3 h; Extended release 6-12 h.

Adverse Effects

- With conventional tablets: Nausea, anorexia, vomiting, asthenia, head ache, pain, dizziness, pyrosis gastralgia, diarrhoea and constipation.
- With extended-release tablets: Asthenia, headache, pain, dizziness, nervousness, tremor, diarrhoea, hypoglycemia and flatulence.
- Glipizide in fixed combination with metformin hydrochloride: Upper respiratory tract infection, diarrhoea, dizziness, hypertension, nausea/vomiting, musculoskeletal pain, headache, abdominal pain and urinary tract infection.

Special Populations

- Renal or hepatic impairment may increase serum glipizide concentrations and reduce elimination.
- Severe renal impairment may decrease the renal excretion and increase the terminal elimination half-life of glipizide metabolites.

Storage

Tablets (conventional)

Tight, light-resistant containers at a temperature <30°C.

Tablets (extended-release)

- Store at 15–30°C.
- Protect from moisture and humidity.

Interactions for Glipizide

Drug	Interaction
Alcohol	Rarely, disulfiram like reactions
Antifungals	Increased plasma concentrations of glipizide and hypoglycemic effect
Anticoagulants, oral	Glipizide could displace or be displaced by oral anticoagulants from plasma protein binding sites
β-Adrenergic blocking agents	Impaired glucose tolerance; increased frequency or severity of hypoglycemia and hypoglycemia-induced complications
Calcium-channel blocking agents	May exacerbate diabetes mellitus
Chloramphenicol	Increases hypoglycemic effect
Cimetidine	Inhibits the hepatic metabolism of glipizide and potentiate hypoglycemic effect
Contraceptives, oral	May exacerbate diabetes mellitus
Corticosteroids	May exacerbate diabetes mellitus
Diuretics, thiazide	May exacerbate diabetes mellitus
Estrogens	May exacerbate diabetes mellitus
Hydantoins	Glipizide could displace or be displaced by hydantoins from plasma protein binding sites

Isoniazid	May exacerbate diabetes mellitus
MAO inhibitors	Increases hypoglycemic effects
Niacin	May exacerbate diabetes mellitus
NSAIDs	Glipizide could displace or be displaced by nonsteroidal anti-inflammatory agents from plasma protein binding sites
Phenothiazines	May exacerbate diabetes mellitus
Phenytoin	May exacerbate diabetes mellitus
Probenecid	Increases hypoglycemic effects
Rifampin	May exacerbate diabetes mellitus
Salicylate	Does not displace salicylate from plasma protein binding sites
Sulfonamides	Glipizide could displace or be displaced by sulfonamides from plasma protein binding sites
Sympathomimetic agents	May exacerbate diabetes mellitus
Thyroid agents	May exacerbate diabetes mellitus

Actions

- Sulfonylurea anti-diabetic agent.
- Lowers blood glucose concentration in diabetic and non-diabetic individuals.
- Stimulates secretion of postprandial endogenous insulin from the beta cells of the pancreas.
- During prolonged administration, extrapancreatic effects such as enhanced peripheral sensitivity to insulin occur and reduction of basal hepatic glucose production may contribute to the hypoglycemic action.

7. POLYMER PROFILE[13]

BP Gelatin

Synonyms Byco; Crysogel; E441; gelatin; instagel; kolatin; solugel; vitagel.

Chemical name Gelatine [9000-70-8]

Empirical formula and molecular weight

Gelatin is a generic term for a mixture of purified protein fractions obtained either by partial acid hydrolysis (type A gelatin) or by partial alkaline hydrolysis (type B gelatin) of animal collagen. Gelatin may also be a mixture of both types.

The protein fractions consist almost entirely of amino acids joined together b amide linkages form linear polymers, varying in molecular weight from 2000 – 200000.

Functional category

Coating agent; Film forming agent; Gelling agent; Suspending agent; Tablet binder; Viscosity- increasing agent.

Description

Gelatin occurs as a light-amber to faintly yellow-colored, vitreous, brittle solid. It is practically odorless and tasteless and is available as translucent sheets, flakes and granules, or as a coarse powder.

Solubility

Practically insoluble in acetone, chloroform, ethanol(95%), ether, and methanol. Soluble in glycerin, acids and alkalis. Strong acids or alkalis cause precipitation. In water, gelatin swells and softens, gradually absorbing between 5 to 10 times its own weight of water. Gelatin is soluble in water above 40˚C, forming a colloidal solution, which gels on cooling to 35–40˚C. This gel–sol system is thixotropic and heat reversible, the melting temperature being slightly higher than the setting point; the melting point can be varied by the addition of glycerin.

Pharmaceutical applications

- Gelatin is widely used in a variety of pharmaceutical formulations, including its use as a biodegradable matrix material in an implantable delivery system.
- It is used to form either hard or soft gelatin capsules.
- Low-molecular-weight gelatins are used to enhance the dissolution of orally ingested drugs.
- Gelatin used in the preparation of pastes, pastilles, pessaries and suppositories.
- It is used as a tablet binder and coating agent and as a viscosity-increasing agent for solutions and semisolids.
- Therapeutically, gelatin has been used in the preparation of wound dressings and has been used as a plasma substitute.
- Absorbable gelatin is available as sterile film, ophthalmic film, sterile sponge, sterile compressed sponge and sterile powder from sponge.
- Gelatin sponge has haemostatic properties.
- Gelatin is also widely used in food products and photographic emulsions.

8. MATERIALS AND METHODS

8.1. LIST OF DRUGS AND EXCIPIENTS

The list of drugs and excipients, their manufacturer and use in the present study are shown in Table 3

Table 3 - List of drugs and excipients

Name of the material	Name of the company	Use in the formulation
Glipizide	Arvind Remedies, Chennai	Active Ingredient
Gelatin type B	Bafna Pharma, Chennai	Polymer
Liquid Paraffin	Merck Laboratories, Mumbai	Dispersion phase
Glutaraldehyde	Merck Laboratories, Mumbai	Cross linking agent
Span 80	Merck Laboratories, Mumbai	Stabilising agent
Sodium hydroxide	IRP ,Chennai	Reagent
Potassium dihydrogen ortho phosphate	Merck Laboratories, Mumbai	Reagent
Petroleum Benzene 60 -80˚ C	Nice Laboratories, Cochin	Solvent

8.2 EQUIPMENTS

Table 4- Equipments used in the formulation and evaluation of Microspheres

Name of the equipment	Name of the company
Magnetic stirrer	Remi, India
Electronic Balance	Asha Scientific Company, Mumbai
UV Spectrophotometer	Shimadzu, Japan
Hot air oven	Industrial heaters, Chennai
Centrifuge	Remi Equipments, India
Light microscope	Focus Trademark, India
Overhead stirrer	Remi, India
Sonicator	Lark, India
SEM Analyser	Hitachi S-3400, Japan
FT-IR Spectrophotometer	Nicolet, India
Dissolution Apparatus	Veego, Mumbai

8.3 PREFORMULATION STUDIES [39]

The preformulation studies are the first step in the rational development of any formulation. It can be defined as "investigation of physical and chemical properties of drug substance alone and combined with the excipients ".The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass produced. The goals of the study are,

- To establish physical characteristics.
- To establish its compatibility with the excipients.
- To determine kinetic rate profile.

8.4 DRUG – POLYMER COMPATIBILITY STUDIES

8.4.1 Fourier Transform Infra Red Spectroscopy[40]

The compatibility between pure drug and polymer was detected by FT-IR spectra obtained .1-2 mg of glipizide alone, mixture of drug and polymer were weighed and mixed properly with potassium bromide uniformly. The spectra's were recorded over the wave number 4000-500cm^{-1}.

Table 5- Composition of drug and excipients for FTIR spectra

S.No	Ingredients
1.	Drug
2.	Gelatin
3.	Drug + Gelatin mixture

8.5 CALIBRATION CURVE FOR GLIPIZIDE[41]

8.5.1 Preparation of pH 7.4 Phosphate Buffer

An accurately measured 50ml of 0.2 M potassium dihydrogen orthophosphate was transferred to a 200ml volumetric flask and 39.14ml of 0.2M sodium hydroxide was added to it. Volume was made up to 200ml with distilled water, mixed and pH was adjusted to 7.4 with 0.2M sodium hydroxide or 0.2M orthophophoric acid.

8.5.2 Preparation of 0.2 M Potassium Dihydrogen Phosphate

An accurately weighed 27.21g of monobasic potassium dihydrogen phosphate was dissolved in 1000ml of distilled water and mixed.

8.5.3 Preparation of 0.2 M Sodium Hydroxide Solution

An accurately weighed 8g of sodium hydroxide pellets were dissolved in 1000ml of distilled water and mixed.

8.5.4 Standard Curve in Phosphate Buffer pH 7.4

An accurately weighed amount of 100 mg of Glipizide was transferred into a volumetric flask and volume was made up to 100 ml with 7.4 pH phosphate buffer. The resulted solution had the concentration of 1mg/ml which was labelled as stock solution 1. From this stock solution 10 ml was taken and diluted to 100ml with 7.4 pH phosphate buffer which was given the solution having the concentration of 100µg/ml which was labelled as stock 2.

Necessary dilutions were made by using second solution to give the different concentration of glipizide2-10µg solutions can be made.

The volumetric solution 10µg/ml was scanned in a UV-Visible double beam spectrophotometer to determine the λ max of the drug. The absorbance of the volumetric solution was recorded at λmax of 223nm and plotted graphically to the standard graph of glipizide.

8.6 PREPARATION CROSS LINKED GLIPIZIDE LOADED GELATINMICROSPHERES [42,43,44]

Gelatin microspheres were prepared by an emulsification cross linking method. 10 ml of 15% (w/v) aqueous gelatin solution preheated to 60˚C. The specified quantity of Glipizide was dissolved in phosphate buffer pH 7.4 preheated to 60˚C. Then the mixture was added drop wise to 50 ml of liquid paraffin with 1% w/v span 80 preheated to 60˚C and emulsified by stirring with magnetic stirrer at rpm 1000. Then the stabilized emulsion is allowed to cool and cross linking agent glutaraldehyde was added and the stirring was continued at room temperature for 6 hours. The cross linked microspheres were cooled and washed with Petroleum benzene to remove unreacted glutaraldehyde and liquid paraffin. After washing, the microspheres were dried at room temperature and stored in dessicator.

Table 6-Formulation table

Batch No	Glipizide (mg)	Gelatin (g)	Liquid Paraffin(ml)	Glutaraldehyde(ml)	Drug: Polymer ratio
F1	100	2.0	50	0.5	1:20
F2	100	1.5	50	0.5	1:15
F3	100	1	50	0.5	1:10
F4	100	0.75	50	0.5	1:7.5
F5	100	0.5	50	0.5	1:5
F6	100	0.25	50	0.5	1:2.5

8.7 EVALUATION OF MICROSPHERES[44]

8.7.1 Percentage Yield

The total amount of dried microcapsules was weighed and the percentage yield was calculated by taking into consideration the total weight of the drug and polymer used for preparation of microspheres.

$$\text{Percentage Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

8.7.2 Estimation of Drug Content

100 mg of microspheres was weighed and suspended in phosphate buffer pH 7.4. The suspension was suitably diluted with phosphate buffer pH 7.4 in 100 ml standard flask and filtered to separate the fragments. Drug content was analyzed after suitable dilution by UV spectrophotometer at a wavelength of 223 nm against phosphate buffer pH 7.4 as blank. All the studies were carried out in triplicate.

8.7.3 Entrapment Efficiency (EE)

50 mg of microspheres were powdered and dissolved in phosphate buffer pH 7.4 in 50 ml volumetric flask and made up to the volume. The solution was kept for 1 hour with occasional shaking. Further 1 ml solution was diluted up to 50 ml with phosphate buffer pH 7.4. The content was analyzed spectrophotometrically at 223 nm against phosphate buffer pH 7.4 as blank. The % EE of each formulation was calculated using the following equation

$$\% \text{ EE} = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

8.7.4 Drug Loading Capacity [48]

Drug loaded microspheres were digested with phosphate buffer pH 7.4 at room temperature for 12 h. After filtration and suitable dilution, Glipizide present in the solution was determined.

$$\text{Drug loading (\%)} = \frac{\text{Actual drug content in weighed quantity of powder}}{\text{Weighed quantity of the microspheres}} \times 100$$

8.7.5 Particle Size Analysis [45]

Particle size analysis was carried out by using optical microscopy. About 200 microspheres were selected and their size was determined by using optical microscope fitted with standard micrometer scales. All the studies were carried out in triplicate.

8.7.6Scanning Electron Microscope [44]

Morphological examination of the surface of microspheres was performed using a scanning electron microscope (SEM) (Hitachi S-3400, Japan). The sample was sputtered with gold and the observations were made under vacuum.

8.8 EVALUATION OF FLOW PROPERTIES[45,46,47]

Flow properties of microspheres were investigated by determining by following standard procedures. All studies were carried out in triplicate (n=3).

8.8.1Bulk Density

Bulk density was determined by taking a known weight of dried microspheres in a measuring cylinder and tapping 3 times from 1 inch height at 2 second interval. The bulk volume is noted and the bulk density was calculated from the following equation.

$$\text{Bulk density} = \frac{\text{Weight of microspheres}}{\text{Bulk volume of microspheres}}$$

8.8.2Tapped Density

Tapped density is the ratio of mass of microspheres to the volume occupied by the same mass of the powder after a standard tapping of a measure. Weighed quantity of microspheres was taken in a cylinder and tapping 300 times from 1 inch at 2 second interval. The tapped volume is noted and the tapped density was calculated from the following equation

$$\text{Tapped density} = \frac{\text{Weight of microspheres}}{\text{Tapped volume}}$$

8.8.3Hausner's Ratio

Hausner's ratio is used for predicting the flow characteristics.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

8.8.4Compressibility Index

Compressibility index was determined by using bulk density and tapped density.

$$\text{Compressibility index (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Table 7 - Compressibility index table

Compressibility index(%)	Hausner's ratio	Angle of repose (degrees)	Type of flow
< 10	1.00-1.11	25–30	Excellent
11-15	1.12-1.18	31–35	Good
16-20	1.19-1.25	36–40	Fair
21-25	1.26-1.34	41–45	Passable
26-31	1.35-1.45	46–55	Poor
32-37	1.46-1.59	56–65	Very poor
>38	>1.60	>66	Very very poor

8.8.5Angle of Repose

A funnel was fixed to a stand and bottom of the funnel was fixed at a height of 3 cm from the plane. Microspheres were placed in funnel and allowed to flow freely and the height and radius of the heap of microspheres was measured.

$$\tan \theta = h / r$$

Where, 'h' is the height of heap and

'r' is the radius of heap of microspheres.

8.9 SWELLING RATIO[48]

50 mg of microspheres was placed in a glass vial containing 10ml of phosphate buffer of pH 7.4 at 37± 0.5˚C in incubator with occasional shaking. The microspheres were periodically removed, blotted with filter paper and their changes in weights were measured during the swelling equilibrium was attained. Finally, the weight of the swollen microspheres was recorded after a period of 4 h and the swelling index was calculated from the formula. The studies were carried in triplicate.

$$\text{Swelling Ratio} = \frac{(W_e - W_o)}{W_e}$$

Where, W_o is the initial weight of microspheres and

W_e is the weight of the swollen microspheres at equilibrium with swelling medium at time't'.

8.10 *IN VITRO* MUCOADHESIONSTUDY[49]

The microspheres were placed on the sheep intestinal mucosa after fixing to the polyethylene support. The mucosa was then placed in the dessicator to maintain at > 80% RH at room temperature at 30 min to allow the polymer to hydrate and to prevent drying of the mucus. The mucosa was then observed under a microscope and the number of particles attached to the particular area was counted. After 30 minutes, the polyethylene support was introduced into a plastic tube cut in circular manner and held in an inclined position at an angle of 45˚. Mucosa was washed thoroughly at flow rate of 1 ml min^{-1} for 5 min with phosphate buffer pH 7.4. Tissue was again observed under a microscope to see the number of microspheres remaining in the same field area. The adhesion number was determined by the following equation

$$N_a = N/N_o \times 100$$

Where, N_a is adhesion number,

N_o is total number of particles in a particular area and

N is number of particles attached to the mucosa after washing.

8.11 *IN-VITRO* DRUG RELEASE STUDY[50]

In-vitro drug release studies were performed using USP dissolution test apparatus I (basket type). The dissolution studies were performed in 900 ml dissolution medium (Phosphate buffer pH 7.4), at 50 rpm maintained at 37 ± 0.5ºC. At predetermined time intervals an aliquot of 10 ml was withdrawn and replenished with fresh medium. Amount of drug in each aliquot was determined using a UV-Spectrophotometer (UV-1800, Shimadzu, Japan) at 223 nm using a suitable blank. All trials were conducted in triplicate and the average (± SD) reading was noted.

8.12RELEASE MECHANISM[51,52]

Experimental data were fit to following kinetic equation to determine the order and mechanism of drug release.

Zero order equation

$$C = k_o t$$

First order equation

$$\text{Log } C = \text{Log } C_0 \, kt / 2.303$$

Higuchi's square root model

$$Q = Kt\sqrt{t}$$

Where, ´Q_t´is amount of drug released at a time't',

Q_o is the initial amount of drug in the dissolution medium,

k, K, K_{HC} are release constants.

Hixson Crowell cube root law

$$Q_0^{1/3} - Qt^{1/3} = K_{HC}t$$

Korsemeyer-Peppas empirical power law

$$M_t / M_\infty = K\, t^n$$

Where, M_t/M_∞ is the fraction of drug released at a time't',

K is the Korsemeyer release constant and 'n' characterizes the mechanism of drug release from formulations during diffusion process

.

n = 0.45 for case I or Fickian diffusion, n < 0.5 for anomalous or non- Fickian diffusion, n = 1 for case II transport and n < 1 for super case II transport.

8.13 COMPARATIVE STUDY WITH MARKETED SAMPLE [23]

In-vitro drug release studies were performed using USP dissolution test apparatus I (basket type). The dissolution studies were performed in 900 ml dissolution medium (Phosphate buffer pH 7.4), at 50 rpm maintained at 37 ± 0.5ºC. At predetermined time intervals an aliquot of 10 ml was withdrawn and replenished with fresh medium. Amount of drug in each aliquot was determined using a UV-Spectrophotometer (UV-1800, Shimadzu, Japan) at 223 nm using a suitable blank. All trials were conducted in triplicate and the average (± SD) reading was noted.

The *invitro* drug release in phosphate buffer pH 7.4 for the optimized formulation was compared with the marketed sample using a similar procedure (SR tablet).

8.14 STABILITY STUDY[53]

From the prepared microspheres the formulation which showed optimum drug content and percentage release was selected for stability studies. The optimized formulation were placed in borosilicate screw capped glass containers and stored at a temperature of (40±2° C / 75% RH) for a period of 90 days. The samples were assayed for drug content at regular intervals of 30^{th}, 45^{th} and 90^{th} day.

9. RESULTS AND DISCUSSION

9.PREFORMULATION STUDIES

9.1 Drug-Polymer Compatibility Studies

9.1.1 FTIR Spectroscopy

The compatibility between drug and polymer was confirmed by using FTIR spectroscopy.

Infrared spectroscopic analysis for drug (Glipizide), Gelatin, Drug-gelatin mixture was carried out.

The principal IR peaks of pure Glipizide and Gelatin are shown in table 8

Table 8- FTIR spectrum interpretation

Sample	Characteristics bands	Possible Functionalities
Glipizide	3000-3700 cm^{-1} 2700-3300 cm^{-1} 1650-1700 cm^{-1} 1650-1700 cm^{-1} 1334-1442 cm^{-1} 1157 cm^{-1} 650-900 cm^{-1}	NH- Stretching C-H stretching C=O stretching -CONH stretching C-H bend (Cyclohexane) S=O stretching C-H bend (Benzene)
Gelatin	1689 cm^{-1} 1527 cm^{-1} 1157 cm^{-1}	C=O stretching vibrations N-H bending vibrations C-N stretching vibrations

FTIR Spectra of Glipizide

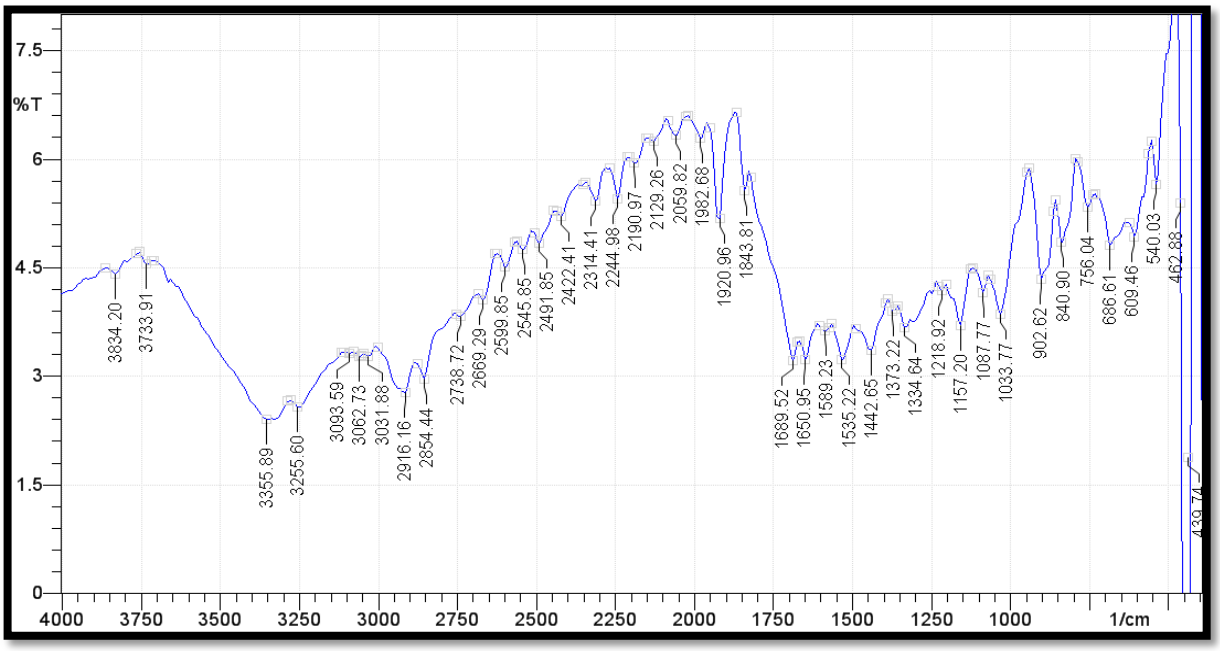


Figure 9

FTIR Spectra of Gelatin

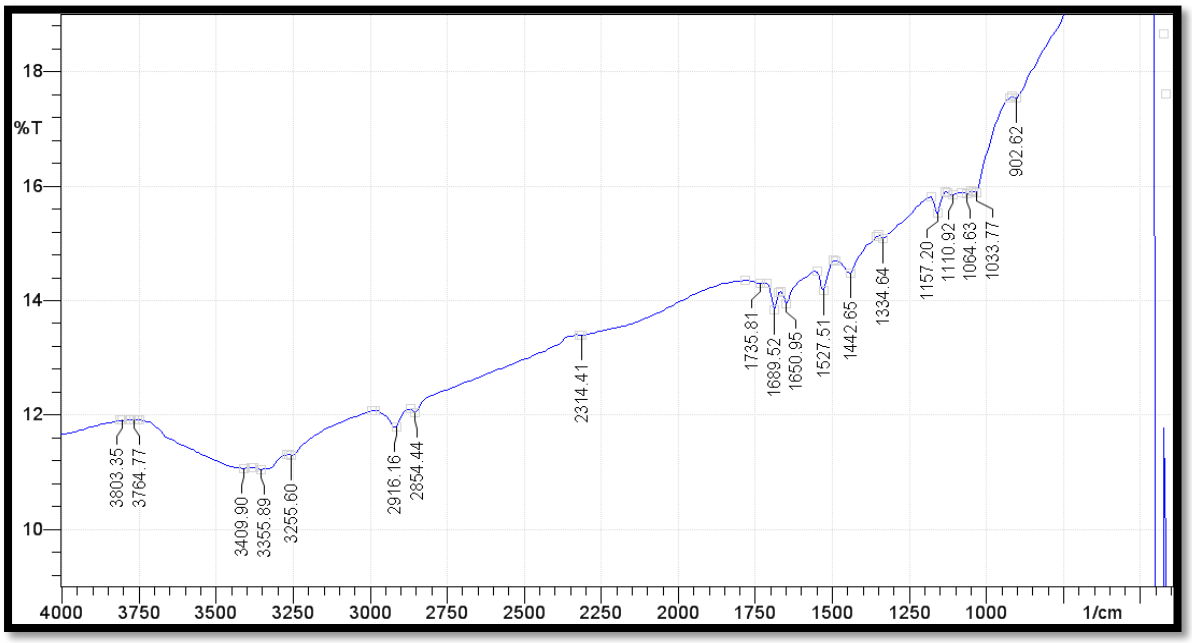


Figure 10

FTIR Spectra of Physical Mixture

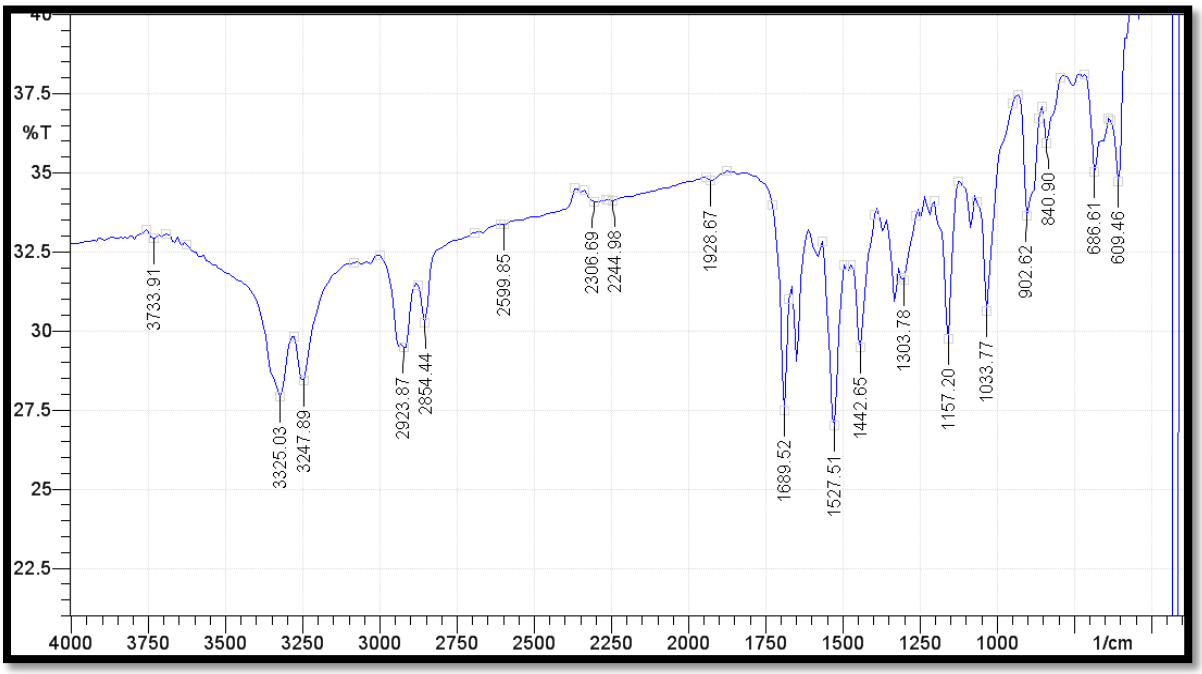


Figure 11

FTIR Spectra of Optimized Formulation

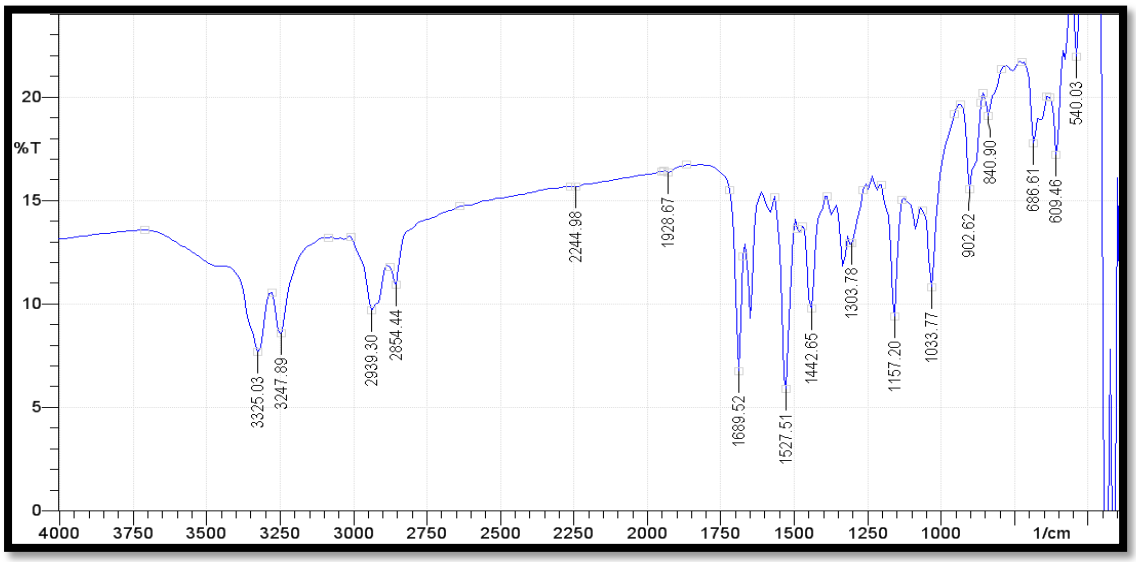


Figure 12

Inference

The peaks observed in the FTIR spectrum of physical mixture and optimized formulation showed no shift and no disappearance of characteristic peaks of drug as well as polymer. This suggests that there is no interaction between the drug and polymer. Hence it can be concluded that the drug maintain its identity without undergoing any chemical interaction with Gelatin.

9.2 STANDARD CALIBRATION CURVE FOR GLIPIZIDE

The UV spectrophotometric method was used to standardize Glipizide at a wavelength of 223 nm. The standard graph was constructed using phosphate buffer pH 7.4.

Table9-Standard Curve for Glipizide

Concentration (µg/ml)	Absorbance
0	0
2	0.123
4	0.230
6	0.338
8	0.452
10	0.565

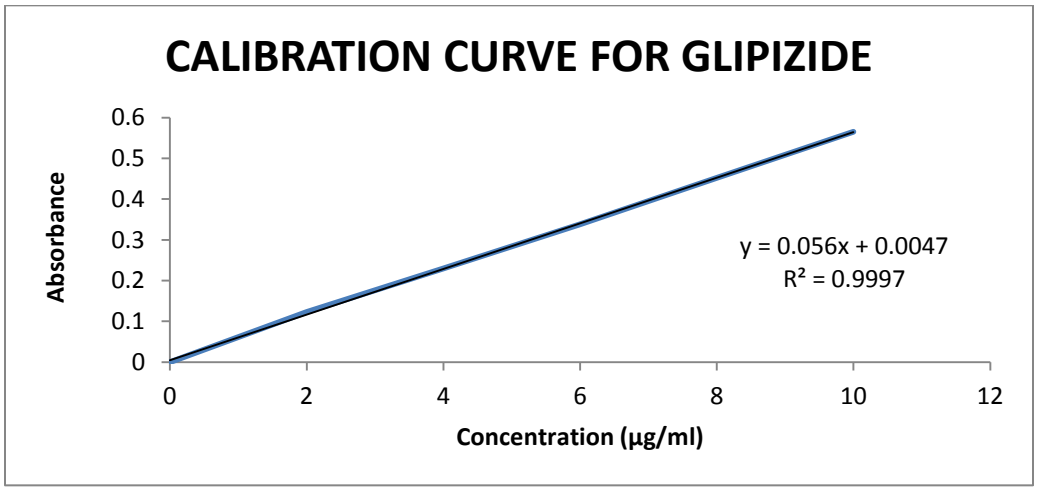


Figure 13- Standard graph of Glipizide in phosphate buffer pH 7.4

Inference

As shown in figure the linearity was exhibited at a concentration range of 0 – 100 µg/ml of Glipizide. It obeys Beer-Lambert's law.

9.3 EVALUATION OF MICROSPHERES

9.3.1 Percentage Yield

Table 10- Percentage yield

Formulation	Theoretical yield (g)	Practical yield (g)	Percentage yield (%)
F_1	2.1	1.950	92.85
F_2	1.6	1.447	90.43
F_3	1.1	1.057	96.09
F_4	0.850	0.797	93.76
F_5	0.600	0.576	96
F_6	0.350	0.280	80.14

After the preparation of microspheres practical yield and percentage yield were calculated. It was found that percentage yield was in the range of 80.14 % to 92.85 %

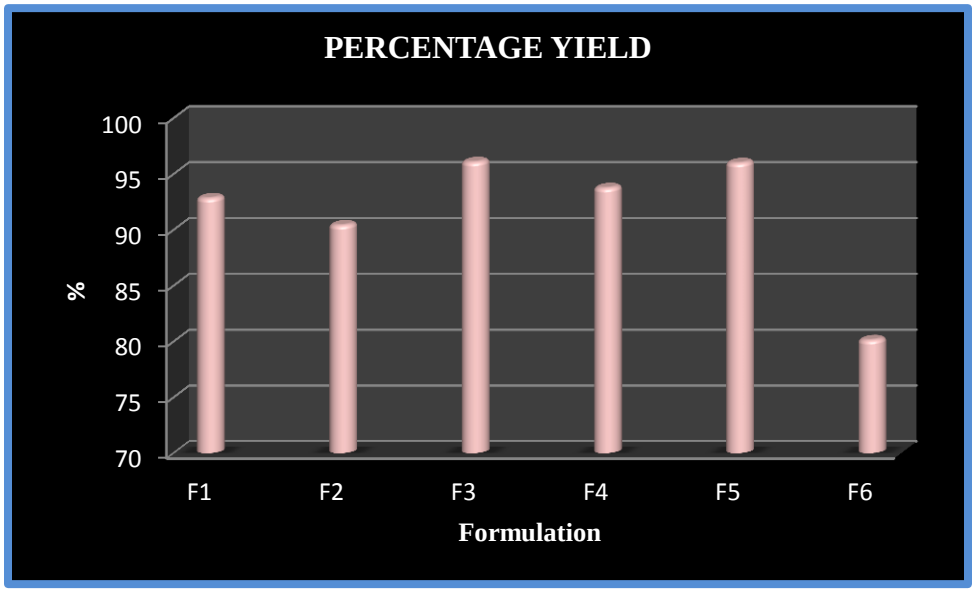


Figure 14- Percentage yield

9.3.2 Drug Content

The drug content was found in the range of 81 to 87.35% w/w.

Table 11-Drug content

Formulation	Drug content (%) w/w
F_1	81.76
F_2	85.75
F_3	87
F_4	83.20
F_5	87.35
F_6	82.84

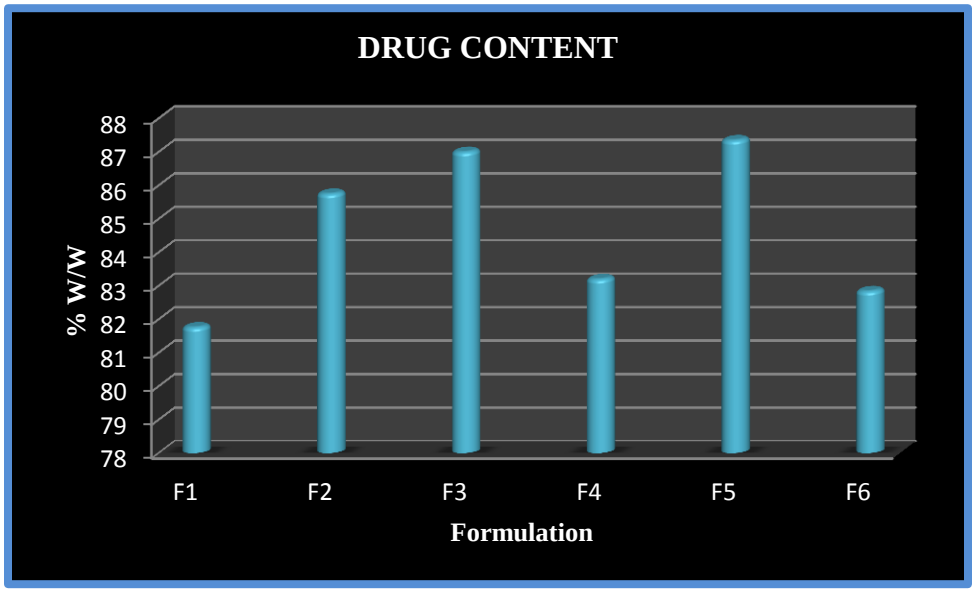


Figure 15- Drug content

9.3.3 Entrapment Efficiency

The results of entrapment efficiency of the microspheres are given in table 12.

Table 12- Entrapment efficiency

Formulation	Entrapment Efficiency (%)
F_1	81
F_2	86.25
F_3	87
F_4	83.25
F_5	87.5
F_6	82

The percentage entrapment efficiency calculated for all microspheres ranged from 81 to 87.5 %. The highest entrapment efficiency is found for the formulation F5.

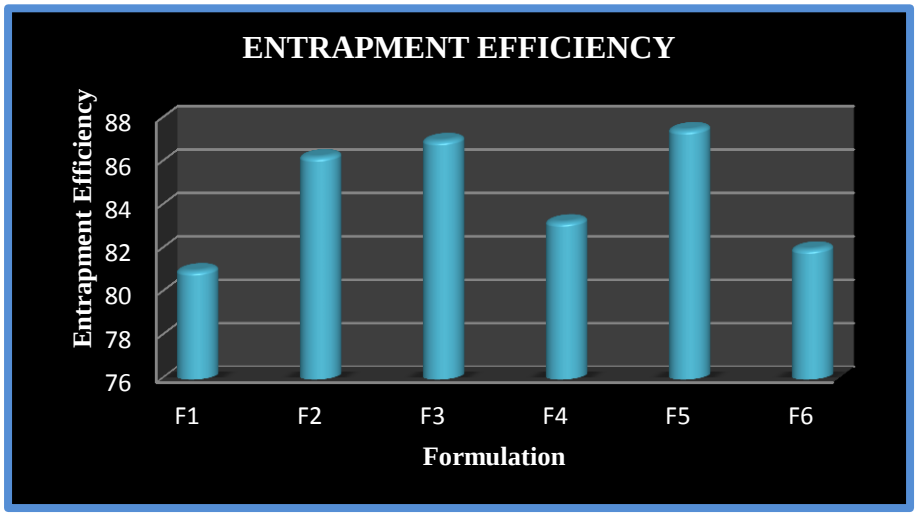


Figure 16- Entrapment efficiency

9.3.4 Drug Loading Capacity

The drug loading capacity of the microspheres was found to be in the range 3.893 to 17.56%.

Table 13- Drug loading Capacity

Formulation	Drug loading Capacity (%)
F_1	3.893
F_2	5.358
F_3	7.56
F_4	10.23
F_5	17.56
F_6	12.39

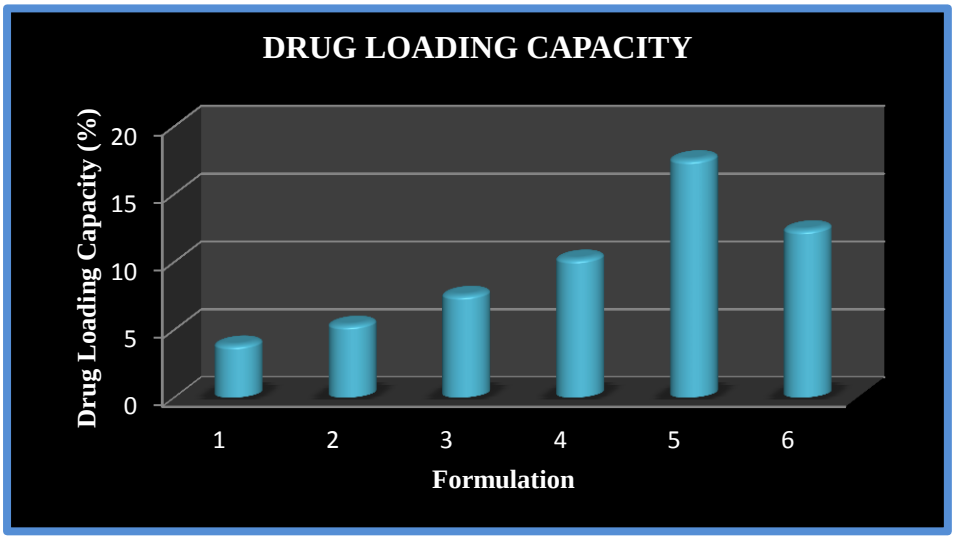


Figure 17- Drug loading Capacity

9.3.5 Mean Particle Size by Microscopy

The particle size was found in the range of 520 to 752µm.

Table 14 - Mean Particle size

Formulation	Mean Particle Size (µm)
F_1	587
F_2	570
F_3	642
F_4	693
F_5	752
F_6	525

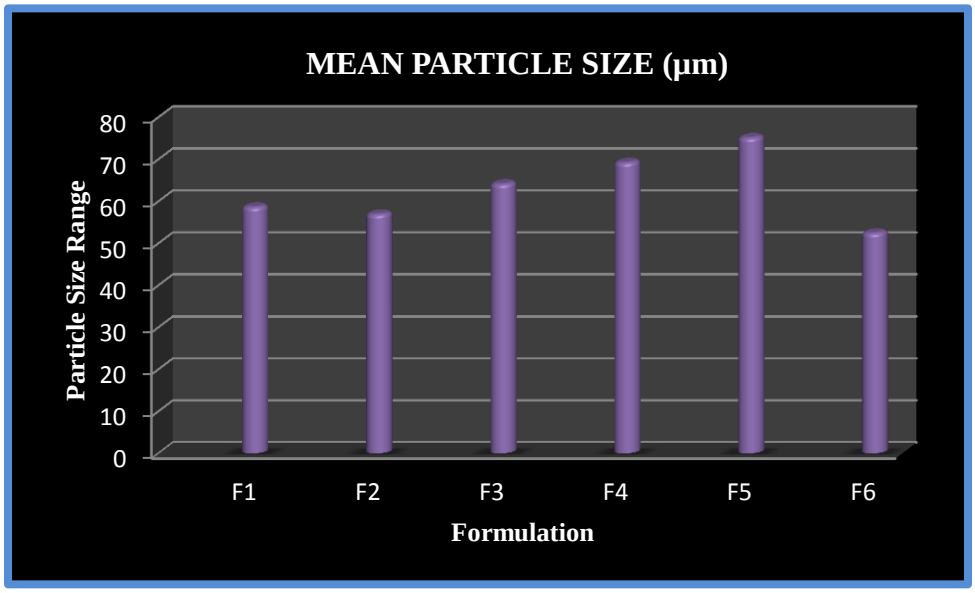


Figure18- Mean Particle Size of Microspheres

9.3.6 Scanning Electron Microscopy

Morphological analysis of the microspheres was carried out using optical microscopy and scanning electron microscopy (SEM).

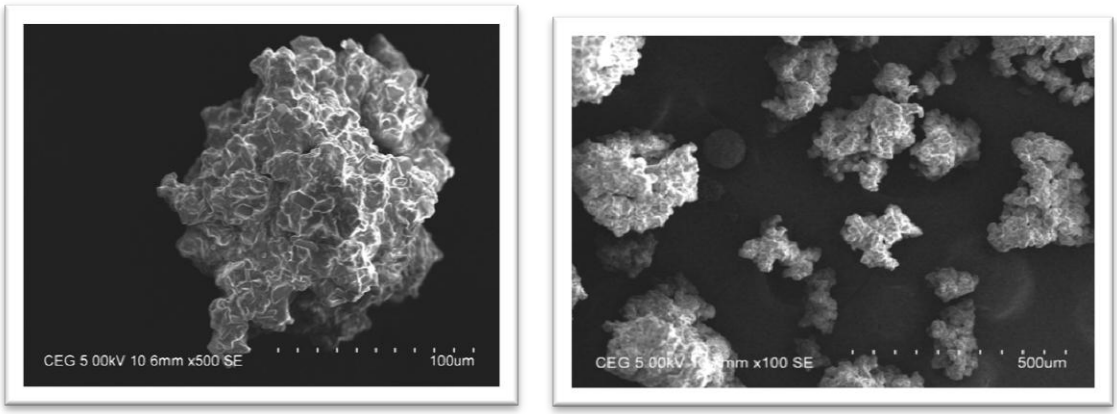


Figure 19- SEM Photographs of optimized formulation F5

Inference

The SEM photograph reveals that the microspheres were discrete and almost spherical in shape. The particle size was found below 500µm.

9.4 EVALUATION OF FLOW PROPERTIES

Table 15- Flow Properties of Glipizide microspheres

Formulation	Bulk density(g/ml)	Tapped density(g/ml)	Angle of repose(˚)	Compressibilityindex(%)	Hausner's ratio
F_1	0.6±0.154	0.75±1.125	26.46± 3.3894	20± 1.25	1.25±0.1857
F_2	0.625±2.15	0.714±1.84	26.83± 0.3412	12.46± 2.01	1.142±0.2103
F_3	0.7±1.267	0.8±2.54	24.61± 1.456	12.5±1.01	1.142±0.9577
F_4	0.627±0.145	0.718±0.115	28.82± 1.270	12.47±1.78	1.143±0.1245
F_5	0.7±2.12	0.777±0.255	26.2±2.8202	9.09±1.23	1.11±0.2134
F_6	0.71±0.850	0.83±1.12	25.54± 2.3810	12.36±0.9997	1.141±0.3988

9.4.1 Bulk Density

The bulk density was found in the range of 0.6 to 0.71 g/ml.

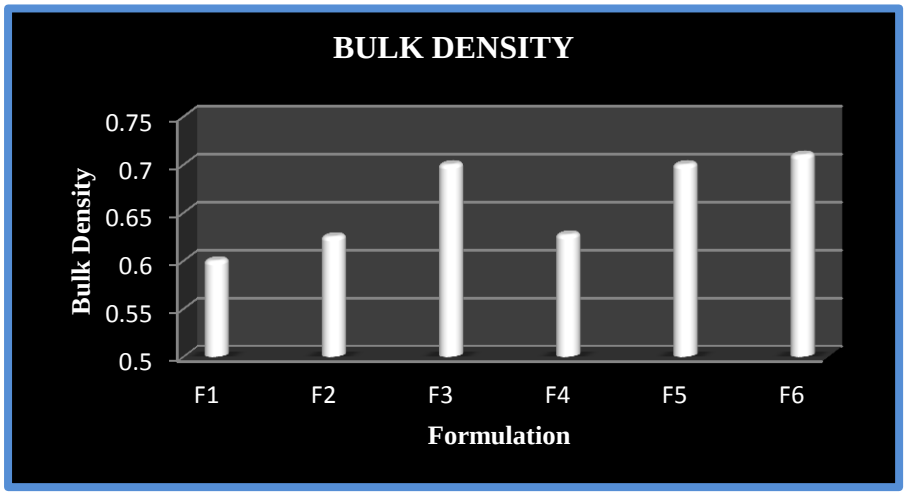


Figure 20- Bulk density

9.4.2 Tapped Density

The tapped density was found in the range of 0.14 to 0.88 g/ml.

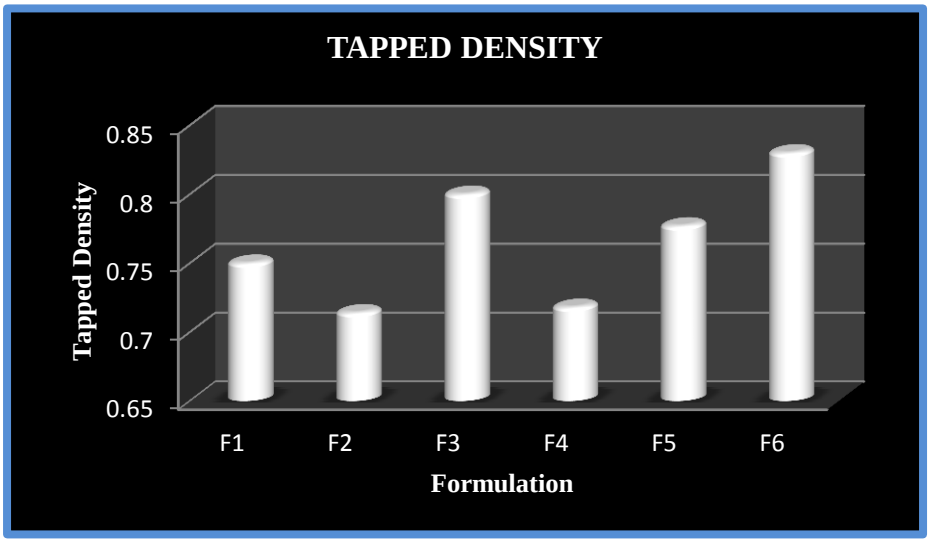


Figure 21- Tapped density

9.4.3 Hausner's Ratio

The Hausner's ratio of the formulations were found in the range of 1.11 to 1.42.

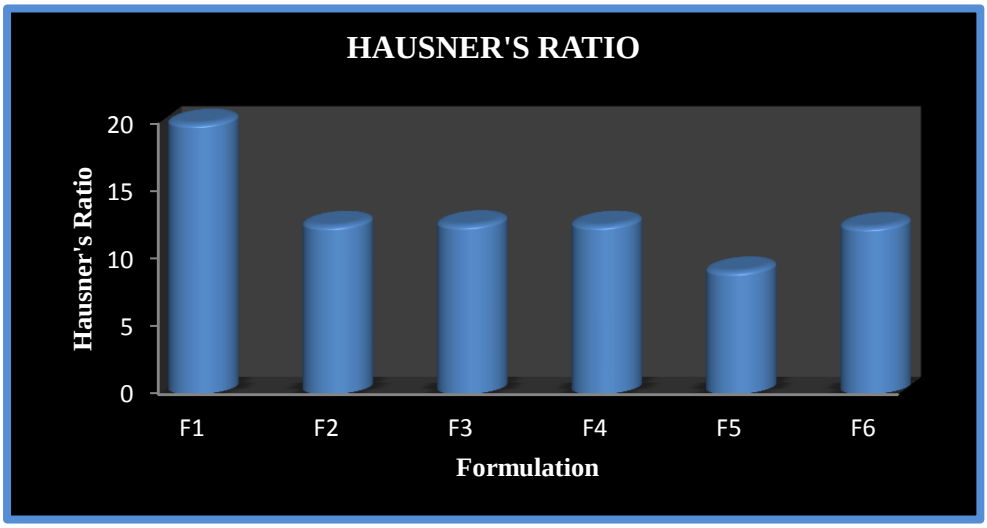


Figure 22- Hausner's ratio

9.4.4 Compressibility Index(%)

The Compressibility index was found in the range of 9.09 to 20 %.

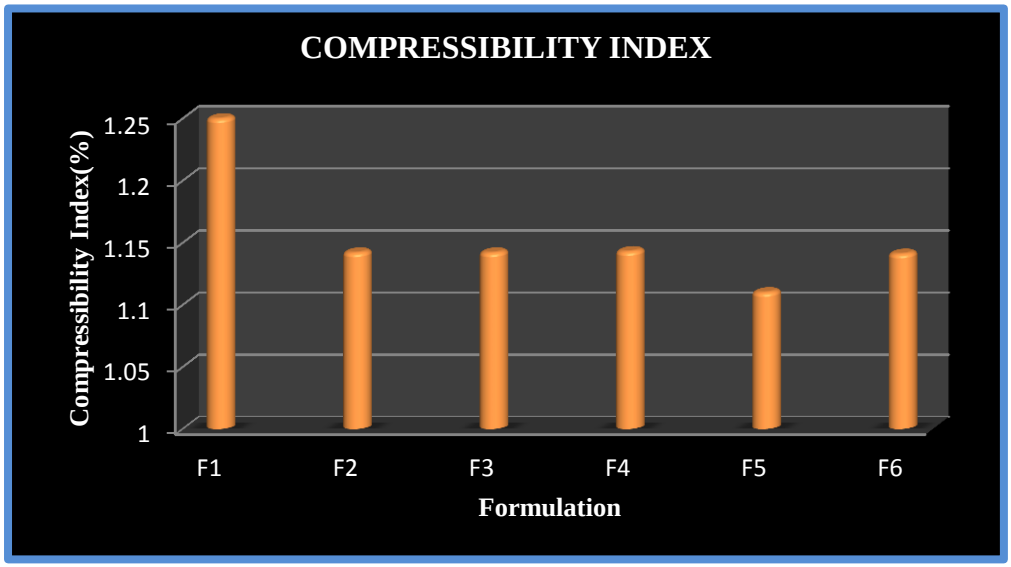


Figure 24-Compressibility index

9.4.5 Angle of Repose

The angle repose was found in the range of 25.54 to 28.8˚. The flow property of the microspheres was found to be good.

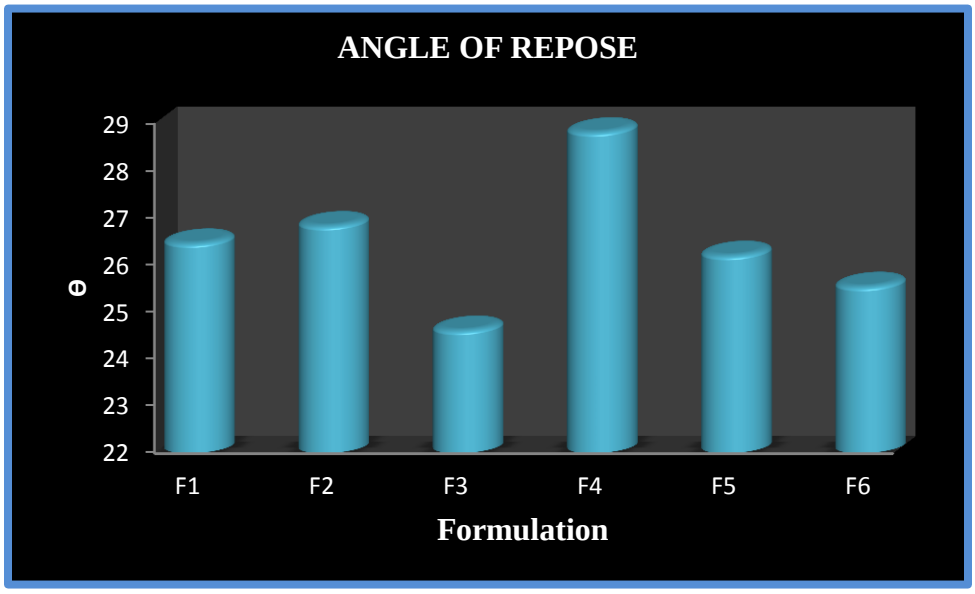


Figure 24 - Angle of repose

Inference

The flow properties of the formulated microspheres were evaluated. The flow property was found to be good.

9.5 SWELLING RATIO

The results of swelling studies of the microspheres are given in table 14.

Table 16- Swelling ratio

Time in hours	F_1	F_2	F_3	F_4	F_5	F_6
0	0	0	0	0	0	0
1	0.8	0.6	0.714	0.94	0.78	0.96
2	1.4	1.04	1.5	1.54	1.64	1.38
3	1.9	1.96	2	2	2.2	2
4	1.9	1.96	2	2	2.2	2
5	1.9	1.96	2	2	2.2	2

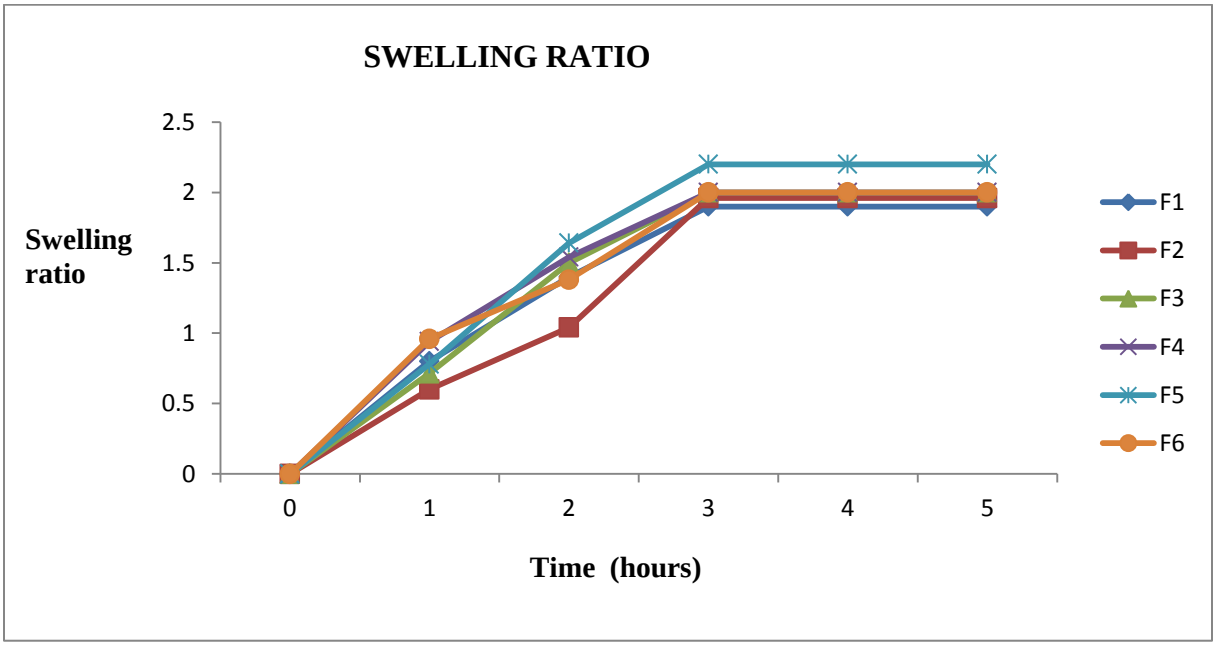


Figure 25- Swelling ratio

9.6 *IN VITRO* MUCOADHESION STUDY

Table 17- *In vitro* mucoadhesion study

Time (hours)	% Microspheres adhered					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
0.5	92.67±1.15	94±2.00	94±2.00	90.67±2.0	95.33±1.15	91.33±1.15
1	86.66±1.15	87.33±3.06	86±2.00	82.67±1.15	88±2.00	81.33±1.15
2	76.67±1.15	81.33±3.06	78.67±2.31	70±2.00	82.67±1.15	74.67±2.31
3	70.01±2.00	72.67±2.3	68.67±1.15	60.67±1.15	75.33±1.15	57.33±1.15
4	56.67±1.15	60.67±1.15	54±2.00	44.67±1.25	64±2.00	40±2.00
5	43.33±1.15	46±2.00	38.67±1.15	33.33±2.01	49±4.16	28.6±1.15

Figure 26-*Invitro* mucoadhesion study

9.7 *IN VITRO* RELEASE STUDY

Table 18 – Cumulative percentage drug release of Formulation F1 to F6

Time (hours)	F1	F2	F3	F4	F5	F6
1	9.88	5.89	9.74	7.29	9.24	9.24
2	18.24	12.1	13.81	15.18	14.55	17.8
3	24.51	17.88	20.2	21.21	24.47	23.21
4	31.51	23.41	27.22	27.85	30.6	31.29
5	37.72	27.86	30.92	36.72	36.8	38.25
6	45.72	34.3	39.75	42.99	45.01	41.56
7	52.07	38.22	44.15	49.29	48.75	52.01
8	79.32	44.9	50.86	57	58.39	58.3
9	86.25	50.67	59.33	61.52	63.57	65.55
10	93.03	55.59	63.89	71.94	71.4	69.94
11	100.75	67.37	67.37	76.6	75.06	74.77
24		74.22	89.65	98.88	100.3	97.7

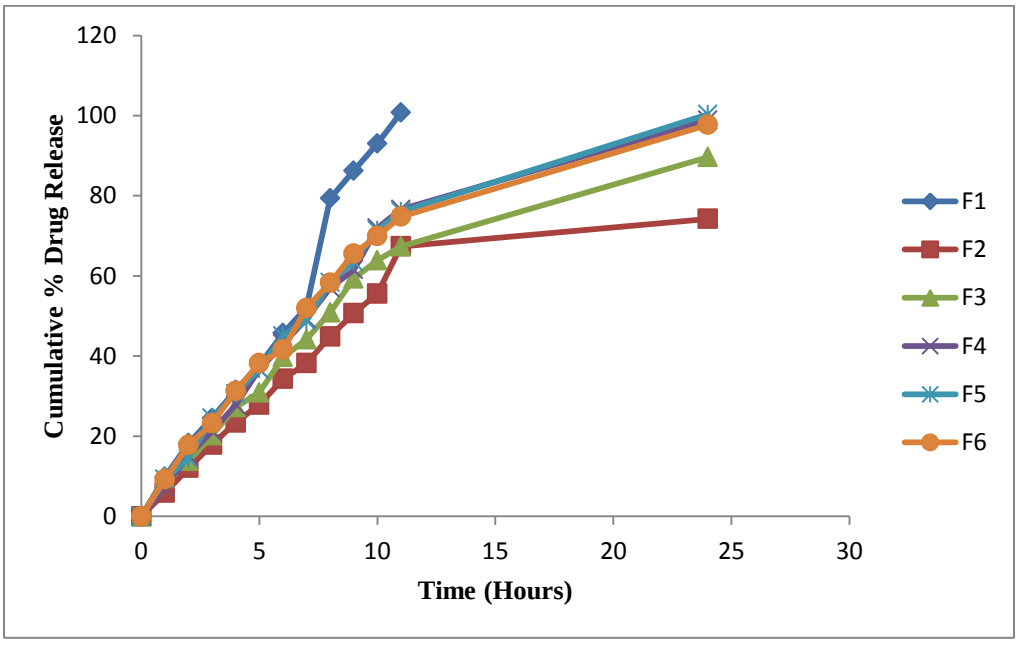


Figure 27-*In vitro* Drug Release Study of Formulations F1- F6

9.8.1 *In vitro* evaluation study of F5 formulation (1:5)

The *in vitro* drug release results are given below

Table 19 - *In vitro* drug release

S.No	Time in hours	Cumulative % drug release
1	1	5.89 ± 1.035407
2	2	12.10 ± 1.00307
3	3	17.88 ± 1.01444
4	4	23.81 ± 0.61822
5	5	27.86 ± 0.42129
6	6	34.30 ± 0.27108
7	7	38.22 ± 0.82890
8	8	44.9± 0.6468
9	9	50.67± 0.80155
10	10	55.59± 0.72997
11	11	67.37± 0.60942
12	12	70.01± 0.59211

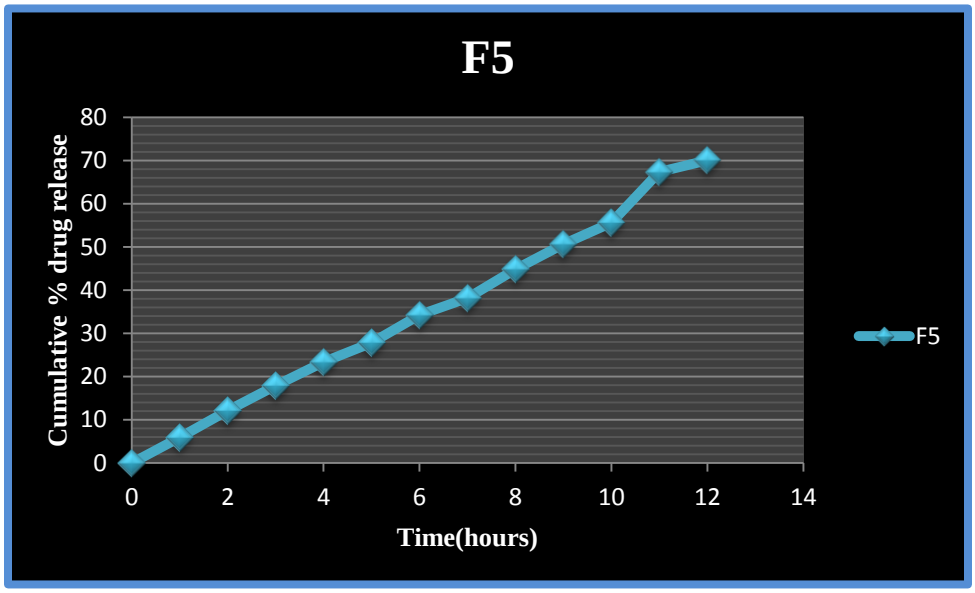


Figure 28-*In vitro* Drug Release Study of Formulation F6

9.9 RELEASE KINETICS AND MECHANISM

The kinetics of drug release for optimized Glipizide loaded gelatin microspheres F5 in phosphate buffer pH 7.4 Table 19

Table 20- Mechanism of release kinetics

Time (hours)	Cumulative % drug release	%Cumulative drug remaining	Log% Cumulative drug remaining	Square root of time	Log time	Log% cumulative drug release	Cube root of % drug remaining
0	0	100	2.000	0	α	α	4.6415
1	5.89	94.11	1.9736	1	0	0.7701	4.5486
2	12.10	87.9	1.9439	1.4142	0.3010	1.0827	4.446
3	17.88	82.12	1.9144	1.7320	0.4771	1.2523	4.3465
4	23.41	76.59	1.8841	2	0.6020	1.3694	4.2467
5	27.86	72.14	1.8581	1.236	0.6989	1.4449	4.1628
6	34.30	65.7	1.7572	2.4494	0.7781	1.5352	4.0351
7	38.22	61.78	1.7908	2.6457	0.845	1.5822	3.953
24	75.06	24.94	1.1368	4.898	1.3802	1.8754	2.9216
28	99.17	0.83	1.2623	5.2915	1.4471	1.9963	0.9397

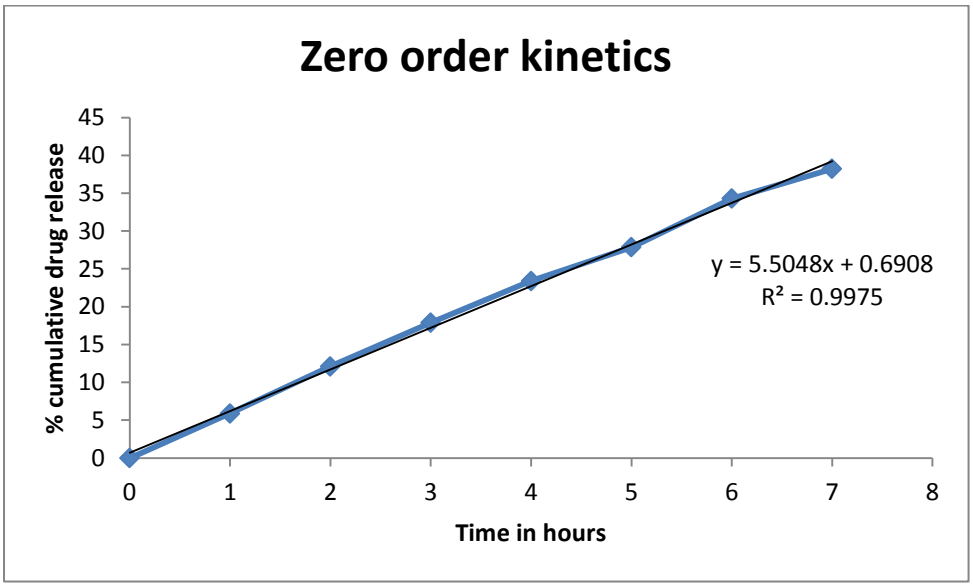


Figure 29- Zero order kinetics for Optimized Formulation F5

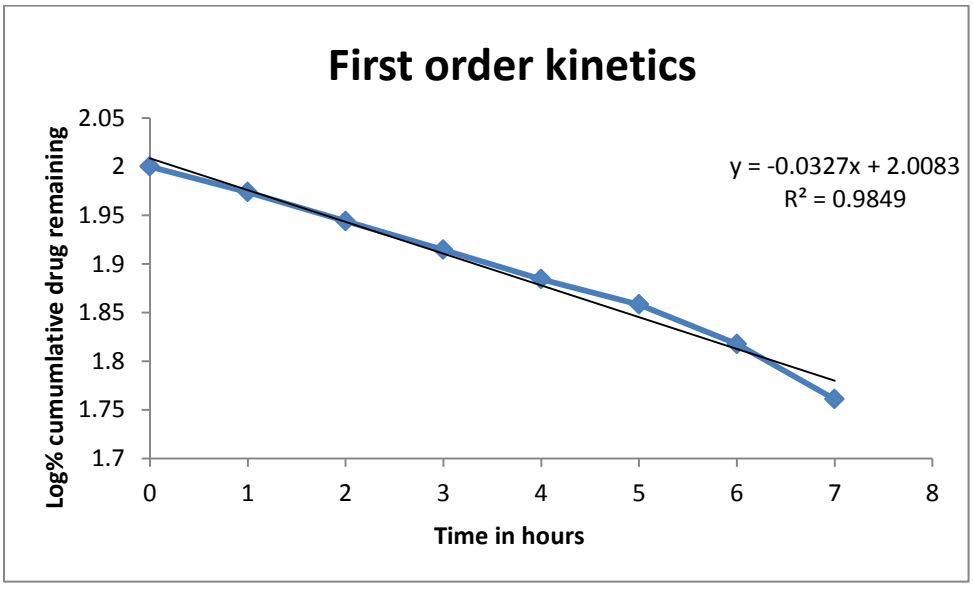


Figure 30 - First order kinetics for Optimized Formulation F5

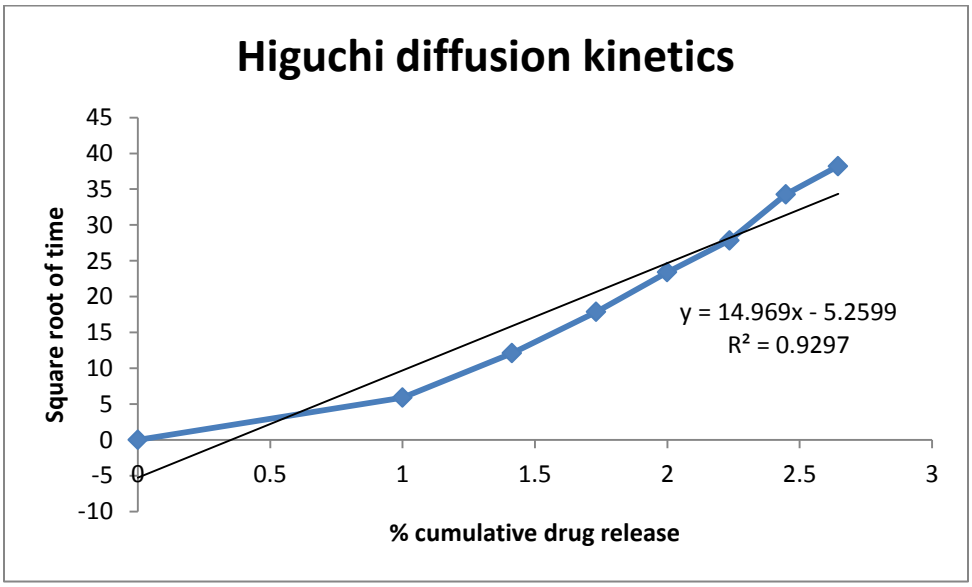


Figure 31- Higuchi diffusion kinetics for Optimized Formulation F5

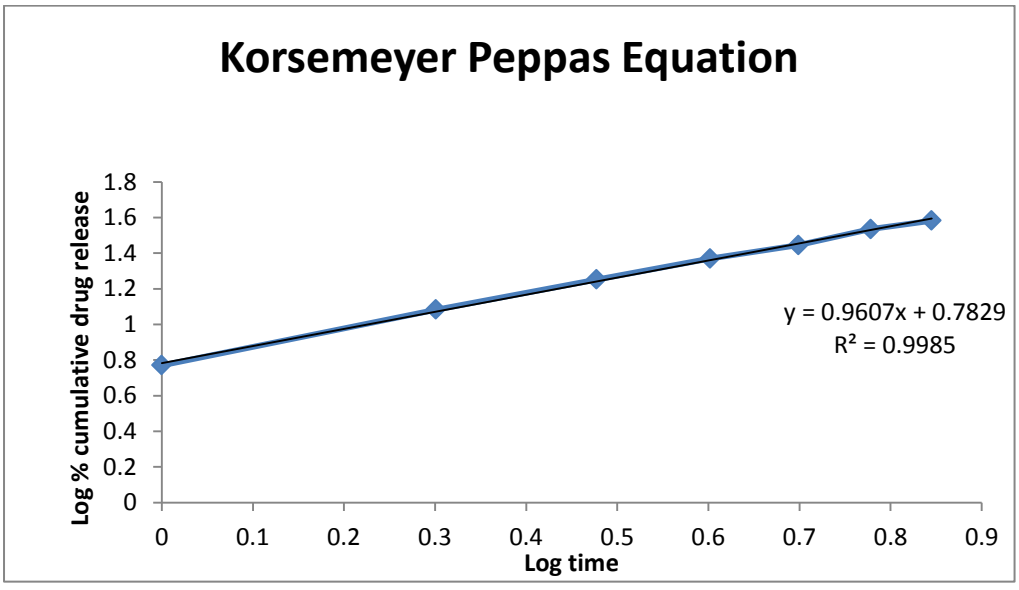


Figure 32-KorsemeyerPeppas Equation for Optimized Formulation F5

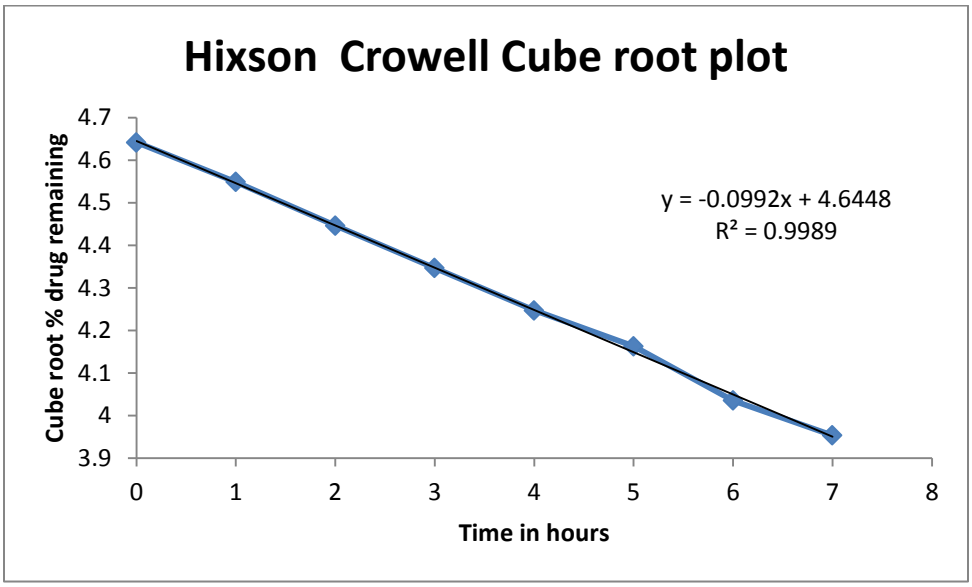


Figure 33-Hixson Crowell Cube root plot for Optimized Formulation F5

- ✓ The order of drug release was found to be zero order, in which regression value was 0.997.
- ✓ The 'n' value of Korsemeyer Peppas equation was found to be 0.998. From this it was concluded that the drug release follows a non-fickinian diffusion.

Inference

Drug can be released from microspheres by the mechanism of diffusion. It was observed that the mechanism governing the release of Glipizide from gelatin based drug delivery system is predominantly drug diffusion.

9.10 COMPARATIVE STUDY WITH MARKETED SAMPLE

The results of the comparative study for SR tablet and optimized formulation F5 in phosphate buffer pH 7.4 are given in the table 18.

Table 21- Comparison study of Marketed SR tablet with Optimized formulation

Time in hours	% Cumulative drug release of SR Tablet	% Cumulative drug release of Optimized formulation
1	8.69 ± 0.61384	5.89 ± 1.035407
2	16.10 ± 0.98266	12.10 ± 1.00307
3	23.23 ± 1.1172	17.88 ± 1.01444
4	30.52 ± 0.62933	23.81 ± 0.61822
5	37.88 ± 0.5309	27.86 ± 0.42129
6	46.42 ± 0.6153	34.30 ± 0.27108
7	51.31 ± 1.60852	38.22 ± 0.82890
24	101.57 ± 0.8890	75.06 ± 0.59211

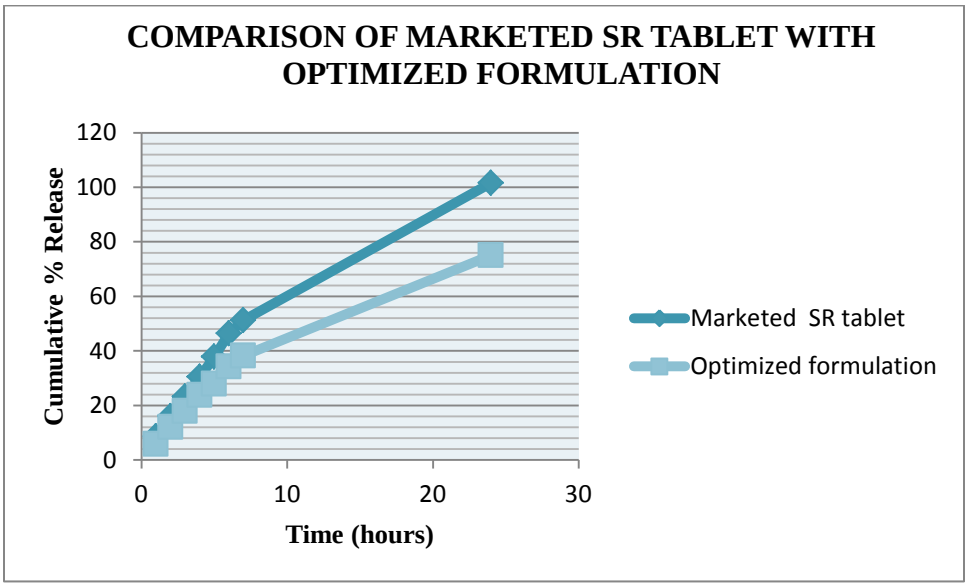


Figure 34- Comparison of Marketed SR with Optimized Formulation F5

9.11 STABILITY STUDY

Stability study was carried out for the F5 formulation by exposing it to a temperature 40 ± 2°C/ 75% RH for 3 months. The sample was analyzed for drug content at the regular intervals. It was found that no remarkable change in the drug content of F5 formulation. This indicates that F5 was stable for following temperature.

Table 22-Stability study data for F5 formulation

S.No	Days	% Drug content (w/w) 40± 2° C/ 75%RH
1.	0	87.35 ± 00
2.	30	81.32 ± 0.041
3.	45	87.18 ± 0.036
4.	90	87.12 ± 0.02

10. SUMMARY

- An attempt was made to formulate glipizide loaded microspheres using Gelatin as a mucoadhesive polymer by emulsion cross linking method using 25%v/v glutaraldehyde as a cross linking agent for management of type 2 diabetes mellitus.
- The literature survey reveals that extensive research has been carried out on development of mucoadhesive microspheres containing anti-diabetic drug by various methods. It is noteworthy that there was no literature review available describing the formulation of glipizide loaded mucoadhesive microspheres prepared using Gelatin hence the study was further preceded.
- In the present study, F1, F2, F3, F4, F5 and F6 formulations were prepared using gelatin as a polymer (1:20, 1:15, 1:10, 1:7.5, 1:5 and 1:2.5) in six different ratios. The effect of polymer as well as decreasing concentration of gelatin on microspheres was studied by subjecting all the formulations to various evaluation parameters.
- The FTIR study was carried out for the drug, polymer, physical mixture and optimized formulation F5. In FTIR study, all characteristic peaks in the spectra appeared without any remarkable changes showing that there is no chemical interaction between the drug and polymer used in the preparation of microspheres.
- The mean particle size study was carried out by using microscopic analysis and found that the range for all formulations was varied from 525 to 792 µm due to change in drug and polymer ratio.
- The morphology of optimized formulation was studied by SEM analysis and found that shape of microspheres were spongy, discrete, almost spherical and found to be satisfactory.
- The drug content for all the formulations was found to be in the range of 81 to 87% w/w. The formulation F5 had the highest drug content of 87% w/w.
- The entrapment efficiency of all formulations was found to be in the range of 81 to 87.35%. The entrapment efficiency of formulation F5 was found to be higher.
- The drug loading was found to be in the range 3.58 to 17.5%.The formulation F5 had the maximum loading efficiency.

- All the microsphere formulations showed equilibrium swelling ratio in the range of 1.9 to 2.2. The formulation F5 had the swelling ratio of 2.2 at the end of 5 h.
- The *in vitro* mucoadhesion study was conducted for all the formulations and the results were found in the range of 28.6 to 49%. The results revealed that the formulation F5 had 49% mucoadhesion at the end of 5h.
- The *in vitro* drug release study was carried out for all the formulations and the formulation F5 (1:5) showed sustained release of 75.06% at the end of 24 h.
- The results of *in vitro* drug release studies showed that the drug release was found to be retarded by increasing the polymer ratio.
- The *in vitro* release study results were applied to various kinetics models to predict the mechanism of drug release.
- The release rate followed zero order kinetics and good linearity was found with Higuchi diffusion kinetics.
- In Korsemeyer Peppas equation, the n value was greater than 0.5 indicating anomalous diffusion or non- fickian diffusion may be the mechanism of release.
- The *in vitro* drug release in pH 7.4 for optimized formulation was compared with the marketed glipizide sustained release tablets and the results showed that the microsphere formulation had a sustained release of 75.06% when compared to marketed sustained release tablet 101.57% at the end of 24 h.
- Results suggest that formulation F5 prepared using drug: polymer ratio 1:5 showed good entrapment efficiency, sustained drug release and satisfactory mucoadhesion as compared to other formulations, so it was further subjected to the accelerated stability study.
- The formulation F5 was stable at the tested storage condition (40± 2˚C/75%RH) up to 3 months. No significant changes were observed in the drug content at the end of 3 months.

11. CONCLUSION

The mucoadhesive microspheres of Glipizide were prepared by Emulsion cross linking method using natural polymer Gelatin utilizing temperature change and cross linking agent glutaraldehyde was able to sustain the drug release efficacy.

By varying the drug: polymer concentration ratio, the drug release was sustained.

The evaluation parameters like morphological analysis, drug content, entrapment efficiency, drug loading capacity, swelling ratio, *in vitro* mucoadhesion studies and *invitro* drug release studies was done for the microspheres and found to be satisfactory.

The stability study results shows that the formulation F5 was stable at temperature 40 ± 2˚C/75% RH at the end of 3 months.

The comparative study with the marketed SR formulation results showed that the F5 microsphere formulation had a sustained and prolonged drug release at the end of 24 h than the marketed glipizide SR formulation.

The results of the study revealed that the use of natural polymer Gelatin is an effective strategy for the designing and development of glipizide loaded mucoadhesive microspheres for easy, reproducible and effective oral controlled drug delivery for the treatment of type II Diabetes mellitus.

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